# UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA

## DOTTORATO DI RICERCA INTERNAZIONALE IN "INSECT SCIENCE AND BIOTECHNOLOGY"

## XXIV CICLO

**MARIANGELA COPPOLA**

# PROSYSTEMIN COORDINATES MULTIPLE RESPONSES IN TOMATO

**Coordinatore**

**Prof. Francesco Pennacchio**

**Relatore**

**Prof. Rosa Rao**

**ANNO ACCADEMICO 2010-2011**

## SUMMARY

Since plants are sessile organisms, they couldn't escape stress conditions so they are obliged to develop defence strategies to protect themselves against all environment threats and all kind of pests. These defences can be always expressed (constitutive defences), or they can be activated by pests attack (inducible defences). Based on the mode of action plant defensive traits are distinguished between direct and indirect. The first includes physical barriers and metabolites interfering directly with pests. Indirect defence involves any plant traits that attract natural enemies of pests, such as predators and parasitoids (Kessler and Baldwin, 2002). Based on their feeding apparatus, insects can be divided in two main group: chewers and phloem-feeders. These two groups determine different damages and consequently different plant responses. Phytophagous arthropods, that cause extensive tissue damages, induce changes in plant gene expression similar to mechanical wounding (Walling, 2009). Damages caused by piercing-sucking insects affect a small leaf area. Using a stylet to pierce cells, aphids consume large quantities of phloematic fluid being in continuous contact with plant cells, usually from hours to weeks. Plant responses against piercing-sucking insects share similarity to pathogen response. Based on the invasion strategy and the mode of feeding, also pathogens can be distinguished in several groups: biotrophic, necrotrophic and hemi-biotrophic. The first group extracts nutrients from living cells infiltrating and establishing their *hyphae* within the cell. Necrotrophic fungi, instead, kill the cell before invasion taking nutrients that are released by damaged tissues. Hemi-biotrophic fungi combine both strategies during their life. Although herbivores and pathogens have different attacking strategy, they share the attitude to release molecules and patterns that can elicit plant responses. The activation of defence responses is controlled by different hormone-regulated pathways that often overlap. While SA is mainly associated to responses against biotrophic fungi, JA and ET are manly involved in responses to insects and necrotrophic fungi. In *Solanaceae*, a family of defense-related peptide hormones called systemins are involved in the activation of defense genes in response to wounding and herbivore attacks (Ryan and Pearce, 2003). Systemin (Sys) is a 18-amino-acid peptide hormone which is initially released at wound sites and represents a primary wound signal in tomato. Sys is released from a cytosolic precursor protein of 200 amino-acid called prosystemin (ProSys) through an

unknown mechanism. The activation of defense genes by systemin is mediated by the octadecanoid signaling pathway (Ryan, 2000). The tomato genome possesses a single copy of the *ProSys* gene that is under tissue, cell-type specific, developmental, and environmental regulation (Ryan and Pearce, 1998). Its over-expression under the regulation of the CaMV 35S promoter in transgenic tomato plants led to the constitutive accumulation of high levels of several defensive proteins in leaves (McGurl *et al.,* 1994). Using the opposite approach, McGurl and collaborators (1992) demonstrated that transgenic tomato plants suppressed in *ProSys* expression were impaired in the accumulation of PIs. Sys is released from its precursor after wounding or herbivory and it binds its receptor, SR-160, starting the depolarization of plasma membrane, alkalinization of apoplast, $Ca^{2+}$ influxes and $H_2O_2$ release. These events activate MAPK signalling and phospholipase A2 (PLA2) that tears out α-linolenic acid (LA) from membrane starting the octadecanoid pathway in which JAs (12-OPDA, JA, MeJA, JA-Ile) are produced. In addition to Sys, tomato plants possess three functionally related hydroxyproline-rich glycopeptides (TomHypSys I, II, and III) of 15–20 residues. ProSys and HypSys work cooperatively in the regulation of defence responses in tomato (Narvaez-Vasquez et al., 2007). The aim of this research activity is to shed more light on ProSys involvement in tomato responses against several stress conditions and to evaluate a possible use of this plant hormone as a broad-spectrum defence instrument. While *ProSys* involvement in signal transduction of responses to wounding and chewing insects have been widely studied during last decades, its effect on phloem-feeders and pathogenic fungi have not been yet characterized. For this reason, tomato cv. “Red Setter” was chosen to over-express *ProSys* gene since this tomato cultivar lacks the dominant allel at *Mi* locus, a gene involved in resistance against aphids, nematodes and white flies (Rossi *et al.,* 1998). *Solanum lycopersicum* cv. “Red Setter” was genetically transformed via *A. tumefaciens* containing the pMZ vector carrying *35S*$^2$*:prosystemin*, already described by Rocco and collaborators (2008). A wide population of transgenic plants expressing *ProSys* at different levels was obtained. Among $T_0$ population three genotypes were selected, for further investigations, according to their *ProSys* expression levels: RSYS24, the genotype expressing *ProSys*, at very high level, RSYS32, which shows an intermediate *ProSys* expression and RSYS17, a co-suppressed genotype in which not only ProSys, but several JA-dependent genes are strongly

down-regulated. The expression analysis of late defence genes located downstream the octadecanoid pathway confirmed the up-regulation of three genes coding for proteinase inhibitors consistent with literature (McGurl *et al.,* 1994; Ryan, 2000). A correlation between *ProSys* and *PIs* expression levels is hypothesized but further investigations on a larger number of replicates are needed to assess a significant quantitative relationship between them and to verify if this quantitative effect is reflected during the steps of octadecanoid pathway. These different *PIs* expression levels are not related to a different effect on *Spodoptera littoralis* larvae weights. In fact, as shown by bioassay, larvae fed on *ProSys* highly over-expressing lines were similarly affected in their nutrition since they showed reduced weights. Surprisingly, larvae fed on the co-suppressed line showed intermediate weights between the two other transgenic genotypes and the control. A time-course expression analysis after *Spodoptera littoralis* feeding helped to explain these findings and to shed more light on ProSys involvement in tomato systemic response. Larvae damages on Red Setter plants induces only locally *ProSys*, while *InhI* is induced both locally and distally. These data are consistent with the hypothesis by Lee and Howe (2003) that Sys locally induces JA synthesis to reach a threshold required for the activation of the systemic response. A systemin-independent *PIs* induction could be correlated to the weak affection of *Spodoptera littoralis* larvae weight observed on the co-suppressed RSYS17 plants by bioassay. *InhI* up-regulation in damaged leaves of highly *ProSys*-expression or *ProSys*-suppression could be easily explained by the release of plant elicitors (OGAs) or insect elicitors during herbivory. Therefore in RSYS17 also the systemic induction of *PIs* could be attributed to a systemin-independent pathway. In RSYS24 the strong anti-herbivore activity could be the sum of the systemin signalling cascade and this independent one that both converge in the activation of JA pathway. The independent pathway could be induced by elicitors present in insect oral secretions or by plant elicitors, like OGAs, but it could be also mediated by the recently discovered hydroxyproline-rich systemin glycopeptides (HypSys) that Narvaez-Vasquez and collaborators (2005) associated to ProSys in the coordination of tomato defence responses. These molecules or new ones could locally trigger the activation of octadecanoid pathway stimulating JA production that reach a threshold required for the systemic response, similarly and independently to systemin activity. JA is then transported

through the plant to activate defence genes in unwounded leaves and it is released to alert neighbours plants. Since *ProSys* over-expressing plants were found to be more attractive to the aphid parasitoid *Aphidius ervi* (Corrado *et al.,* 2007), its involvement in the regulation of direct defence in response to aphids is supposed. ProSys conferred-resistance to aphids and fungi was underlined by bioassays underlining its broad-spectrum role in tomato. The choice of a tomato cultivar lacking any known resistance gene active against aphids, allowed to attribute the strong effect of *ProSys* over-expressing tomato on *M. euphorbiae* longevity and weight exclusively to ProSys. A time-course expression analysis of JA- and SA-pathways involved genes on Red Setter untransformed plants after *M. euphorbiae* infestation underlined a strong induction of *InhI, Kunitz, Lap* and *Threonine deaminase* for JA pathway and *PR1* and WRKY for SA pathway. These data are consistent with recent findings by Li and collaborators (2006) about the induction of both SA and JA pathways in tomato after aphid infestation. The comparison with the expression profiles of the same genes in RSYS24 and RSYS17 lines revealed that ProSys induces most of the genes associated to aphid response except *PR1*, that is considered a marker of the SAR (Walling, 2009). So, ProSys-conferred resistance to aphids is mainly mediated by JA pathway and it is independent from the activation of *PR1*. A similar approach was adopted to explain the strong resistance of *ProSys* over-expressing plants and the intermediate effect of RSYS17 to *Botrytis cinerea.* This analysis underlined two response types in tomato, one mediated by SA and another by JA. Referring to the widely reported antagonism between JA and SA, *ProSys* co-suppression and the consequent down-regulation of JA-related genes activate SA-mediated pathway conferring to RSYS17 plants resistance to *Botrytis cinerea.* Oppositely, RSYS24 resistance is attributed to the up-regulation of *InhI, Pti5* and *arginase*. Since RSYS24 is more resistant than RSYS17 the *ProSys*-induced JA component of tomato defence against *Botrytis* is stronger and more relevant than the SA effect in responses to necrotrophic fungi. The microarray analysis carried out during this research project underlined many defence-related differentially expressed genes, most of them supporting these biological evidences. First of all several genes coding for messengers involved in the early steps of stress responses are induced, such as *calmodulin 1*, *MAP kinase 3 (MPK3), calcium-dependent protein kinase (CDPK), Jasmonate-ZIM domain 3, purple acid phosphatises* and *LRR-*

*domain receptor kinases.* The activation of so many signals could pre-alert these plants in the rapid activation of defence responses. Most of the genes involved in early and late steps of JA pathway are induced as well as several class of PR proteins and SA-regulated genes consistent with recent microarray dataset about Arabidopsis responses to the cabbage aphid *Brevicoryne brassicae* (Kusnierczyc *et al.,* 2008) and to *Botrytis cinerea* (Asselbergh *et al.,* 2001). Moreover, *ProSys* over-expression up-regulates a large group of genes involved in oxidative stress such as NADH reductase, gluthadione-S-transferase, peroxidases, hydrogen peroxide-induced protein and tioredoxin. Several auxin-related transcripts that were up-regulated in RSYS samples may trigger the increase of ROS production, as demonstrated by Boyko and collaborators (2006) and Kawano (2003). Divol and collaborators (2005) reported the up-regulation of wall-associated enzymes after *Mizus persicae* infestation on *Apium graveolens*. Wall-associated enzymes are also induced in *Botrytis*-infested tomato (Asselbergh *et al.,* 2001). The expression of these defence components were modulated in RSYS plants showing up-regulation of pectin methylesterase and several other enzymes involved in the phenylpropanoid pathway. This pathway is responsible of the production of many anti-microbial and anti-fungal compounds and is required for cell wall reinforcement (Naoumkina *et al.,* 2010). ProSys induction of phenylalanine ammonia lyase (PAL), caffeic acid O-methyltransferase (CAOMT ) and ferulic acid hydroxylase (FAH), caffeoyl-CoA Omethyltransferase (CCoAMT ) shares similarity with results previously described (Bhuiyan et al.,2009) about wheat defence to powdery mildew. Another interesting group of differentially regulated sequences have been associated to abiotic stress responses supporting recent findings from our lab (Orsini *et al.,* 2010). Ultraviolet hypersensitive 3 (UVH3), several class of pathogenesis-related proteins, annexin 3-11 that have been associated to drought stress responses (Clark *et al.,* 2010), low temperature and salt responsive elements, late embryogenesis abundant proteins that are involved in lignifications and salt stress tolerance in potato (Park *et al.,* 2011), the GH3-like protein and microtubule-associated protein are all induced by *ProSys* over-expression. The pathway analysis carried out with Paintomics underlined several enzymes coded by ProSys-regulated genes in the arginine and proline biosynthesis. Polyamines (putrescine, sperimine, spermidine) are compatible solutes involved in abiotic (Hussein *et al.,* 2011) and biotic (Larher *et al.,* 2003) stress responses. As

expected for a transgene insertion with a constitutive expression, the impact on metabolism is very strong as indicated by the annotation of many sequences in primary and secondary metabolisms. Consistent with previous phenotypic observations of BBS plants by McGurl and collaborators (1994), RSYS plants showed a stunted phenotype probably due to the high energetic demand for the continuous production of defensive compounds. This hypothesis was supported by Corrado and collaborators (2011) investigations about plant fitness affection by *ProSys* over-expression. The microarray analysis carried out on the RSYS transgenic lines allow to extend the attribution of this stunted phenotype also to the down-regulation of key genes involved in gibberellins biosynthesis that are known to be involved in internodes elongation. These evidences suggest a possible hormonal participation in control of the reduced size of *ProSys* over-expressing plants in combination with the high energetic request due to the constitutive activation of defence responses. All these findings were strongly enriched in their power of knowledge thanks to the study of protein-protein interactions. The production and the proper use of this type of information is of crucial importance in order to understand cell behaviour and, in the case of study, cell organization during defence responses. The PPIs analysis improves the information level obtained through the microarray analysis collocating predicted RSYS differentially expressed proteins in the network of plant proteins involved in the regulation of defence responses. This is a way to get a wider overview of ProSys impact on tomato transcriptome through the prediction of the interactions between proteins coded by the ProSys differentially expressed sequences. Moreover, the players of the correlation between different hormone-regulated pathways can be identified. Since a tomato interactome is not yet available and despite phylogenetical distances between *Arabidopsis* and tomato that belong different families, the the shifting to the *Arabidopsis* interactome was necessary and let to improve the annotation level for the tomato differentially expressed genes. RSYS differentially expressed sequences were converted in their nearest Arabidopsis homologous protein referring to Meir and collaborators (2010). The RSYS network obtained with various bioinformatic tools contains 2066 proteins and among them 195 were from RSYS list showing a good representation of the differentially expressed sequences. This network was evaluated by statistical analysis compared to thousands random networks showing the same size observing a strong clustering

attitude and underlining about 30 hubs, considering as hub nodes with a degree higher than 20 (Aragues *et al.,* 2007). The collapsing approach helped to simplify some areas of the network allowing the biological interpretation of several hubs. The network produced helped to connect many defence components before believed as far in their activity finding more supports to the biological observations. *ProSys*-regulated proteins in RSYS network are often involved in interactions with several class of kinases, receptors and $Ca^{2+}$-related proteins. Moreover, the interaction with many players of oxidative burst as well as with other signalling molecules are observed. A fairly representative group of TFs were up-regulated in RSYS plants such as WRKY, bZIP, MYB, GRAS, TGA and Ap2Erf. All these TFs interacts with calmodulin 1 (CAM1), that could be defined as the best example of node with a polyvalent control in plant defence. From this study ProSys role in the activation and the coordination of a network of proteins involved in plant defence against biotic and abiotic stresses emerged. Exploring RSYS network many interesting interactions between old and new characters of plant defence response are discovered allowing the connection between defence aspects before considered far. The transgenic plant population produced represents a suitable instrument to evaluate a possible quantitative effect of ProSys in tomato and to get a greater insight on the crosstalk between different defence pathways. The introduction of systemin/JA mutants and reverse genetic approaches targeting new interesting genes underlined during this study could help to attribute new functions and to evaluate new players of tomato defence responses.

# 1. INTRODUCTION

The following research activity have been performed at Department of Science of Soil, Plant, Environment and Animal Productions (DISSPAPA) of University of Naples “Federico II”. The aim was to shed more light on prosystemin, a tomato hormone involved in defences against biotic agents, through biotechnological and “omics” approaches. The research project was widely articulated and was carried out in collaboration with the Department of Entomology and Agrarian Zoology “Filippo Silvestri” for insect assays and with the Department of Arboriculture, Botany and Plant Pathology for fungi assays.

## 1.1 Importance of crop protection

Since plants are sessile organisms, they couldn’t escape stress conditions so they are obliged to develop defence strategies to protect themselves against all environment threats and all kind of pests. The term “stress” refers to factors that can affect negatively plant physiology and fitness. Stress factors can be divided in two big groups: abiotic and biotic stresses. The first one includes all altered environmental conditions like high salinity, drought, dehydration, high and low temperatures, UV light with which plants have to cope. Biotic stress includes all living organisms that can damage plants, i. e. herbivores, insects, viruses, fungi, bacteria and nematodes. Huge crop losses are due to these factors affecting negatively the world food request; this situation will get worse considering the estimating growth of human population for the next years and the consequent increase of food required. The FAO, Food and Agriculture Organization of the United Nations, attributes reductions in yield to both biotic and abiotic stresses. Out of a US$1.3 trillion annual food production capacity worldwide, the biotic stresses caused by insects, diseases and weeds cause 31–42% loss (US$500 billion), with an additional 6–20% (US$120 billion) lost post harvest to insects and to fungal and bacterial rots. Crop losses due to pathogens are often more severe in developing countries (e.g. cereals, 22%) when compared to crop losses in developed countries (e.g. cereals, 6%). Another 6–20% (US$120 billion) is estimated to be lost due to abiotic causes. One of the most significant abiotic stress reducing crop yields is water stress, both water deficit stress (drought) and

excess water stress (flooding, anoxia). Among biotic agents, the 29% of losses estimated for the main “cash crops” are attributed to insects and fungi (Oerke *et al* 2004). It is in this context that the need arises to develop crops which are more resistant to pests attack and to unfavourable environmental conditions. Improved crop management systems based upon genetically improved (high-yielding) cultivars, enhanced soil fertility via chemical fertilisation, pest control via synthetic pesticides, and irrigation were hallmarks of the Green Revolution. The combined effect of these factors allowed world food production to double in the past 35 years (Oerke *et al* 2004). The high sensibility developed from people to health and to the environmental safety, pointed the attention of the scientific community on the development of alternative control strategies to the use of chemical pesticides. One important aspect of the Integrated Pest Management (IPM) is the combination between the minimum use of pesticides and the biological control, that consists in the use of natural enemies and their gene products with the aim to contrast pests and to favourite positive interactions. This approach is applicable and necessary for all relevant commercial crops, such as tomato. Four million ha of Earth surface and 125000 ha in Italy are destined to tomato cultivation. Italy is the sixth tomato producer (6.8 millions tons) in the world and the first one in Europe (FAO’s data updated at 2009). So tomato represents a top crop for Italy and the reducing of losses caused by biotic and abiotic agents is an important purpose for the agrarian community.

Stress responses have been extensively studied due to their economic implications, as they can adversely influence the physiology, growth and crop productivity of plants (Kitsios and Doonan, 2011). Only upgrading the knowledge about plants recognition, perception and responses against different stresses and the crosstalk between them, their conscious usage and fortification will be possible in order to enrich IPM programs.

### 1.2 Co-evolution of defence strategies

The mechanisms used by plant cells to perceive intruders and the reciprocal ability of intruders to escape plant defences have been co-evolving and determine the outcome of microbe and insect interactions with their host plant (Berenbaum and

Zangerl, 2008). During this co-evolution specialist insects became able to locate their host plant and as for generalists, that attack many plants species, evolved strategies to overcome plant physical and chemical barriers. As answer, plants developed several complex defence strategies to survive in a so hostile environment. The origins of plant innate immunity may have evolved from mutualistic or commensalistic interactions of microbes/herbivores and plants (Walling, 2009). The ZigZag model (Jones and Dangle, 2006) indicates 5 phases in this co-evolution (fig. 1).

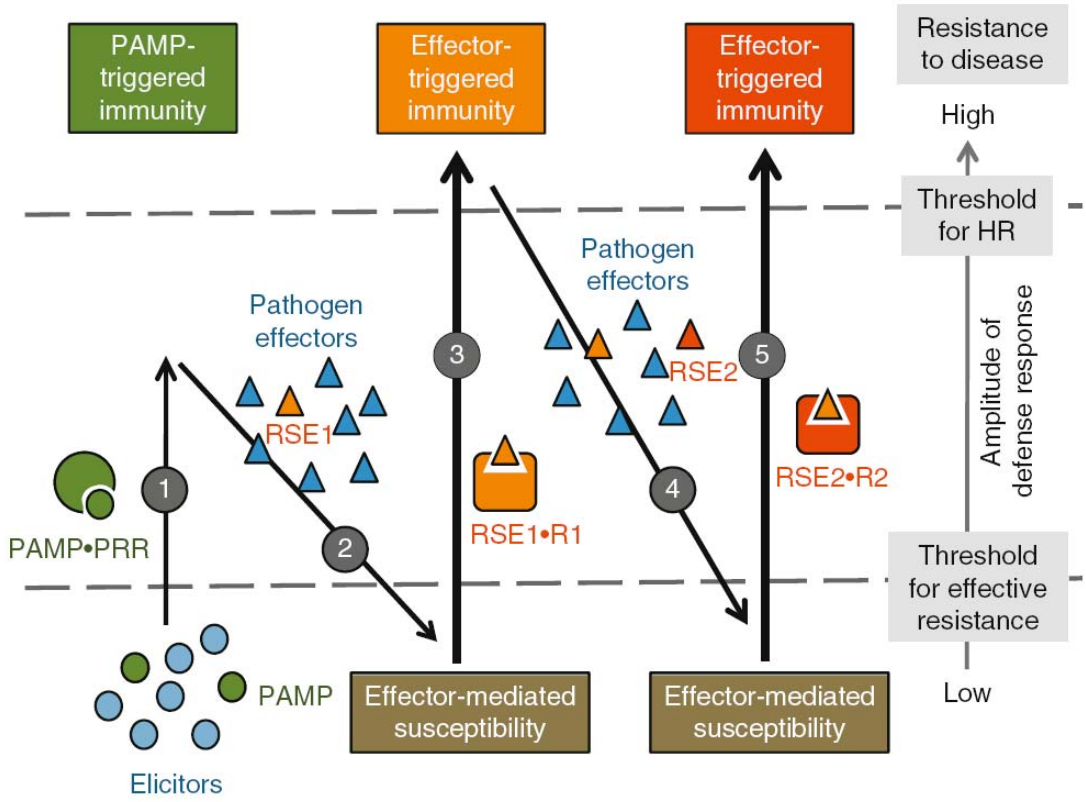


**Figure 1. The ZigZag model (Jones and Dangl, 2006) of innate immunity evolution by Walling L. L. (2009).**

The first one is characterized by the action of constitutive defences and innate immunity. It starts with the recognition of pathogen-associated molecular patterns (PAMPs) and/or herbivores-associated molecular patterns (HAMPs) that include highly conserved proteins, glycolipids, and polysaccharides. These are recognized by pattern-recognition receptors (PRRs) activating the PAMP- or HAMP-triggerd immunity. In the second phase the attacker evolves virulence factors (or race-specific effector, RSE), known to be coded by *Avr* genes, in order to overcome

physical and chemical constitutive barriers. In this way pests and pathogens become able to manipulate innate immunity, determining the successful colonization of the host. This kind of interaction, in which pests are able to damage plant, is defined “compatible interaction”. To answer, plants evolved resistance genes (*R* genes) that recognize *Avr* gene products. In this way, there is the activation of the innate immune system (effector-triggered immunity) to fight pathogen or insect attack limiting symptoms and damage. This situation is defined “incompatible interaction”. In plant-pathogen interactions, this phase mainly consists in the activation of hypersensitive response (HR). This co-evolution goes on in phase four, where pests restore virulence by losing or changing the *Avr* gene, whose product was recognized producing new effectors to action. In phase five, plants responde with the generation of new R gene that recognize and bind the new developed RSEs. The cycling between phases four and five is continuous and reflects the ongoing arms race between plants and their bioagressors. (Walling, 2009).

### 1.3 Plant responses to herbivores

Plants and insects have been coexisting for 350 million years co-evolving through these years. The class Insectia contains more than one million described species and nearly half of them feed on plants (Wu and Baldwin, 2010). Unlike fungi and bacteria, most arthropods that feed on plants have a mobile life stage that allows them to move, evaluate and taste different plant hosts. Insects use tactile, visual, olfactory, and gustatory cues to assess the suitability of their potential host (Walling, 2009). If the plant is not suitable for insect needs, insect can move and choose another host. This choice is strongly influenced by the constitutive defences level, the volatile released and the force and timing of the plant induced responses. The term ‘defence’ describes the pre-existent snags and the induced changes in plant architecture, metabolism and physiology to minimize the negative impact of pests attack. A very clear classification of plant defence traits is proposed by Schaller and collaborators (Schaller, 2008) and summarized in table 1.

**Table 1. Classification of plant defence traits proposed by Schaller and its collaborators (2008).**

| Type of trait | Mode of expression | Mode of action |
|---|---|---|
| Physical | Constitutive | Direct |
| Biochemical | Inducible | |
| Ecological | | Indirect |

### *1.3.1 Plant direct defenses*

Direct defences are any plant traits (e.g., thorns, silica, trichomes, primary and secondary metabolites) that by themselves affect the susceptibility to and/or the performance of attacking arthropods and thus increase plant fitness in environments with herbivores (Kessler and Baldwin, 2002). Tolerance can be considered an element of direct defence and it consists in the decrease of plant fitness during a stress condition. In other words, plants change their rhythm of metabolism, growth and development to cope and survive to the stress factor. The first contact between plant and insect is the surface, so physical barriers are the first obstacle that insect have to overcome. While thorns and spines are effective against mammalian herbivores, thricomes are important players of constitutive defences against insects not only interfering with their movement but also producing repellent substances. The timing of deployment also defines defences (Wu and Baldwin, 2010). Constitutive defences are any pre-existent traits that plants have regardless of stress conditions. Inducible defences are activated after the stress perception and consist in the accumulation of primary and secondary metabolites plus proteins and enzymes coded by defence genes. Since the biosynthesis of defence compounds requires a lot of energy, plants have to balance between defence and growth and development. So, inducible defences are very important for plant since they allow to spend energy only when needed. Also physical barriers can be implemented after stress induction; for example, trichome density increases after insect attack since it is an important interfering element with insect colonization and feeding. Trichome density upgrade may also have a negative effect in plant-insect interactions influencing the abundance and effectiveness of predators and parasitoids feeding on herbivores because of the inhibition of movement and the prolonged searching time. Wax, cuticle and leaf

toughness are other physical components that could affect the preference of insect herbivores during host plant selection (antixenosis), or the performance of the insect on its host plant (antibiosis). Other components of direct defences are primary and secondary metabolites, in part produced costitutively and in part induced. They can affect insect performance altering the nutritional value of their feeding or through a repellent or toxic activity (antibiosis). Plant lectins are a very heterogeneous group that all share one important biological property: they can recognize and bind reversibly to specific carbohydrate structures (Vandeborre *et al.,* 2011). Different carbohydrate structures present in organisms such as viruses, micro-organisms, fungi, nematodes or phytophagous insects were shown to interact with plant lectins (Wong *et al.,* 2010). Chen and collaborators (2007) studied a lectin from tobacco, NIKTABA, that is active against Lepidopteran thanks to its stability to alkaline conditions and proteolysis. These molecules may readily bind the chitin components of insect peritrophic membrane, a protective layer wrapping insect midgut, disrupting and exposing it to toxins and pathogen attacks. Lannoo and Van Damme (2010) proposed lectins as key regulators for various intracellular signalling processes involved in plant stress physiology. Insect damage and wounding strongly induce the production of other chemical compounds showing an excellent anti-nutritional activity, such as tannins. Their oxidation, in fact, promotes the instauration of a wide oxidative stress by peroxidases activity. A rich font of chemicals involved in antibiosis is plant latex, a white sap exuded from leaf damage immediately after herbivory or wounding. There is a lot of evidence to support latex defensive role against herbivores and pathogens by two mechanisms: trapping and immobilizing them due to its stickiness and through its antibiotic activity thanks to its high content of alkaloids, terpenoids, proteinases, chitinases and glucosidades (Konno, 2011). Glucosinolates are a particular class of defence compounds; in fact, in contrast to other defensive compounds, they don't seem to be toxic by themselves but they need to be hydrolyzed to toxic isothiocyanates by myrosinanes. Their sudden release at high levels upon tissue damage is a very effective defence against some generalist herbivores. Instead, several specialist insect species feed without any problem on glucosinolate-containing plants even though they are sensitive to isothiocyanates, and they may even use the presence of glucosinolates in a plant as host recognition cue (Muller *et al.,* 2010). A group of enzymes that can impair

nutrition are polyphenol oxidases and peroxidases that oxidize diphenols producing highly reactively quinones, capable of forming covalent adducts with proteins (Zhu-Salzman *et al.,* 2008). Other defence-involved enzymes are arginase and threonine deaminase that interfere with insect digestion through amino acids degradation. Besides, enzymes that produce superoxide radicals (i. e. NADH oxidases) or hydrogen peroxide (i.e. polyamine oxidases and peroxidases) also work as defence proteins in insect gut. Leucine aminopeptidases (Lap) can be included in this group of defence molecules and are induced by wounding, insect, some pathogens and exogenous JA application as reported by Narváez-Vásquez and collaborators ( 2008). Lap specific function in response to herbivory is still unclear. In tomato, LapA, one of the two major Lap forms, is a modulator of the local and systemic wounding signalling, possibly downstream of JA biosynthesis (Fowler *et al.,* 2009). The most popular anti-nutritive compounds are plant proteinase inhibitors (PIs) which interfere with insect dietary proteolysis inhibiting the access to essential amino acids. Habib and Fazili (2007) classified PIs according to the proteases they inhibit. In tomato, the activation of defence responses involves the synthesis and the accumulation of serin protease inhibitors, the so named *wound-induced protinase inhibitor I* and *II*, with the participation of cystein-, aspartic- and metalcarboxy- protease inhibitors (Ryan, 2000). All these defences can be easily eluded from arthropods. Larvae are mobile and can move to avoid locally induced defences while adult insects may choose on which plant ovipose. Another insect strategy to fight PIs anti-nutritional effect is the compensation, that consists in feeding while a large amount of plant tissue to compensate its poverty due to the high PIs content. Besides, insects can change their set of protease isoforms or change enzyme catalytic domain. An example are the evolutionary changes of a carboxypeptidase of *Helicoperva zea* that is strongly resistant to potato protease inhibitors (Bayes *et al.,* 2005). All these molecules have a direct effect on insect feeding and performance, but each one considered alone is easily overcome by the insect. Only a concerted use of these molecules and other defensive system, such as indirect defences, could be a durable strategy for insects control.

*1.3.2 Plant indirect defence*

Indirect defences are plant traits involved in parasitoids and predators of herbivores attraction. This protection against herbivores is returned by the plant providing nutrients and shelters (i. e. extra-flower nectar and leaf *domatia*). Main mediators of this relatioship are the Volatile Organic Compounds (VOCs), that have been also associated to toxic and repellent effects. For example, Lepidopteran oviposition could be negatively affect by VOCs release in tobacco suggesting their involvement also in direct defences (Kessler and Baldwin, 2002). In plant-insect interaction 4 trophic levels can be underlined: at the first one there is the plant that after insect attack modifies its volatile blend; at the second level there are phytophagous or phloem-feeding insects that choose their host plant; at the third trophic level there are parasitoids and predators attracted by VOCs looking for their prey; at the fourth level there are super-parasites that feed parasitoids.

VOCs are generated by 3 pathways:

- the octadecanoid pathway in which Jasmonic Acid (JA) derivatives and $C_6$ volatile compounds, named “green leaf volatiles” (GLV), are produced. These compounds derive from the degradation of $C_{18}$ fatty acids (linolenic and linoleic acids) in $C_6$ and $C_{12}$ components by hydroperoxide lyase (HPL). $C_{12}$ components are used to produce traumatin, a molecule that is involved in responses against herbivores. Further processing by alcohol dehydrogenase, acetylation, and isomerization leads to the production of the remaining $C_6$-components, like (Z)-3-hexenol, (Z)-3-hexenyl acetate, and the respective E-isomers (Engelberth *et al.,* 2004). GLV emission is induced by wounding and herbivores and they also show anti-microbic and anti-fungal activities (Walling, 2000).
- the shikimate pathway in which methyl-salicylate is produced (MeSA), which is a regulator of pathogenesis-related (PR) genes and systemic acquired resistance (SAR), as salicylic acid (SA). MeSA could also transmits the signal for the activation of defence-related genes to neighbour plants.
- the isoprenoid pathway from which a wide family of compounds, the terpenoids, is produced. Terpenoids derive from 2 precursors: isopentenyl

pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). The first one can be originated from 2 distinct pathways: the mevalonic acid pathway in the cytoplasm or the MEP pathway in the plastids. IPP is isomerized to DMAPP and these two molecules are linked together "head to tail" to form linear chains or they may be arranged to form rings. Based on the number of isoprene unities contained, terpenoids are distincted in: emiterpenes (1 unit), monoterpenes (2 units), sesquiterpenes (3 units) and diterpenes (4 units).

The involvement of VOCs in indirect defences has been widely documented. One of the first discovered example was a study on *Brassica spp.* plants that when infested by *Pieris brassicae* changed their VOCs emissions quantitatively and qualitatively and were more attractive to the parasitoid *Cortesia rubecola* (Kessler and Baldwin, 2002). While terpenoids and other volatiles are released some hours after wounding or insect damages, GLVs emission is very fast. Terpenoids and GLVs are used by aphid predators, such as syrphids, lady beetles, or lacewings, to locate their prey (Verheggen *et al.,* 2008). The volatile blends emitted by different plant species infested by the same herbivore species show large qualitative differences, whereas blends emitted by plants of the same species, but infested by different herbivore species are mostly qualitatively similar with quantitative variation (Dicke, 1999). Volatile blend composition is also influenced by the kind of elicitors released by the insect, following their perception the signal transduction activated. Kessler and collaborators (2006) studied VOCs function as airborne signals between neighbour plants confirming previous observations about this topic. One of the most clear demonstration of this hypothesis was provided by Engelberth and his collaborator (2004). Maize seedlings previously exposed to synthetic and natural VOCs released by damaged plants, resulted pre-alerted to insect attack due to a major accumulation of JA, sesquiterpenes and VOCs emissions. A wider analysis on transcriptome and metabolome modifications caused by airborne interactions between neighbouring plants was carried out by Kessler and collaborator (2006). They studied the priming between damaged sagebrush and native tobacco, finding a higher mortality of *Manduca sexta* larvae on plants that have been previously exposed to clipped sagebrush. They didn't observed a priming involvement in direct defence. Hence, exposure to VOCs from neighbouring attacked plants may allow the neighbours to be pre-alerted and to

respond more rapidly if they are subsequently attacked. In terms of costs, this strategy is very useful to store energies only after insect attack but having resources ready to act since the alarm released by neighbouring plants.

## 1.4 Plant responses to different insect modes of feeding

Based on their feeding apparatus, insects can be divided in two main group: chewers and phloem-feeders. The first one is equipped by grinding mandibles that make them able to destroy a big amount of leaf tissue determining in plant the activation of responses close to those induced by wounding. Since cell and tissue integrity are demolished, chewing insects also encounter the stored defences in vacuoles and trichomes (Morant *et al.,* 2008). Salivary secretions are delivered at the feeding site to lubricate and to solubilise food. Besides, regurgitants are also released by the insect to pre-digest nutrients extra-orally (Walling, 2009). Oral secretions (insect saliva plus regurgitant; OS) contain a big amount of elicitors, molecular patterns, and effectors that trigger direct and indirect defences. Phloem-feeding insects have a stylet with which penetrate the leaf withdrawing cell-content and phloem soups. Despite damaged area is reduced compared to those caused by chewers, aphid activate a wide and varied response. This is not surprising since recent studies assess phloem not only the transport of photosynthesis products, but also the whole-plant communication (Ruiz-Medrano *et al.*, 2001). In a recent review, Kehr (2006) reported that through the phloem several molecules are transported: redox-related molecules (peroxidases, ferredoxin, ion transporters), others involved in calcium signalling (calmodulin, kinases, C2-domain containing protein) and others related to phytohormones (ACC oxidase, ACC synthase, lypoxigenase, allene oxide synthase). Plant responses induced by these two insect modes of feeding and types of damages are different and involve several hormone-mediated defence pathways.

### *1.4.1 Responses to chewing insects*

Chewing insects strongly subtract plant biomass causing a drastic reduction in biosynthetic activity and increase in respiration. Herbivory provokes changes that

substantially overlap with the molecular, physiological, and biochemical responses to mechanical wounding (Walling, 2009). Recently, several studies carried out by Arimura (2008) and Mithofer (2005) workgroups with a mechanical chewer Mecworm investigated on the closeness between herbivores and wounding induced responses. This instrument punches holes in leaves with a similar size frequency of bites of *Spodoptera littoralis* (Egyptian cotton worm) larvae miming responses elicited by this caterpillar. They found that Mecworm causes an increase in JA production and the release of a volatile blend (monoterpenes, sesquiterpenes, homoterpenes, and C6-volatiles) that is qualitatively similar to the blend released by *S. littoralis* larvae. This instrument is very useful to analyze the oral secretions contribute in plant-insect interactions that is still not totally clear. Defense genes and their corresponding proteins can be expressed both locally and systematically, in order to interfere with insect nourishment, oviposition, growth and development. Phytophagous chewing activity produces cell-wall fragments and fatty acids, and causes the mixing of enzymes and substrates from different cellular compartments due to the cell disruption, which alerts the plant of a possible biotic attack. For example, OGAs, which are released from cell wall pectin, are potent elicitors of PAMP- and HAMP-triggered immunity (Walling, 2009). The linolenic acid released from membranes causes the activation of octadecanoid pathway in which jasmonic acid (JA) and its derivatives are produced. Jasmonates regulate a multidimensional defense network to herbivores by increasing secondary metabolites, enhancing emission of volatile blends to attract natural enemies and prime defense, stimulating wound signalling and perception, and increasing the levels of proteins that inhibit or deter insect feeding or growth (Frost *et al.,* 2008; Howe and Jander, 2008). In tomato plants, mechanical damage induces the synthesis of more than 20 defense proteins (protease inhibitors, leucine aminopeptidase and polyphenol oxidase), components of signal transduction pathways and proteases (Ryan, 2000). The anti-nutritional effect of these defense proteins is powerful in some cases, but often a compensatory strategy is applied by insects (par. 1.3.1).

### *1.4.2 Responses against phloem-feeding insects*

Phloem-feeding insects, such as whiteflies, aphids and psyllids, are mainly included in Hemipterans order. Their mouth apparatus is constituted by a stylet used to penetrate the cuticular layer, the epidermis and the mesophyll to reach the phloem bundles in which they suck the fluid rich in nutrients for a time period ranging from hours to weeks. The primary force for stylet penetration and movement is mechanical, but saliva constituents may also enable this process (Miles, 1999). Stylets have two canals: the biggest is for nutrients uptake, while the other one is the salivary canal to delivery water and gelling salivas into the plant. The watery saliva is released at the feeding site through the salivary canal and resorbed through the feeding canal to taste the feeding environment prior to establishing a feeding site (Walling, 2008). Gelling saliva creates a skin around the stylet facilitating its penetration and isolating it from apoplastic defences. The damage generated by this type of insect pests is very different from chewers, given the limited leaf area affected by the bite and given the continued interaction with the plant. Damage entities that phloem-feeding insects can cause are very different so also the consequent expression profiles determined on plant are varied. Genes involved in JA-signalling, oxidative burst and genes involved in secondary and primary metabolism are all affected in their expression. Besides, genes coding for transcription factors, receptors, kinases and other components of signal transduction, in particular leucine-rich receptor-like protein kinases (LRR-RLKs), are regulated. These responses range from extensive overlap with wounding to the promotion of SA-mediated responses (Kempema *et al.,* 2007; Martinez de Ilarduya *et al.,* 2003). In a time-course gene expression analysis after cabbage aphid infestation on *Arabidopsis* plants, Kusnierczyk and collaborators (2008) observed the regulation of genes involved in ROS (Reactive Oxygen Species) production, SA- and JA-mediated pathways, senescence, cell wall organization and camalexin biosynthesis. Also in tomato, the co-expression of JA- and SA- signalling was observed finding a biphasic response: JA-regulated wound-response genes expressed early and genes encoding pathogenesis-related (PR) proteins expressed at later times (Martinez de Ilarduya *et al*., 2003). In contrast, in the greenbug (*Shizaphis graminum*)-sorghum and silverleaf whitefly-Arabidopsis interactions, SA-responsive genes are primarily expressed (Kempema *et al*., 2007).

### 1.5 Plant responses against pathogens

To date, there are more than 10000 fungal species known to cause diseases on plants, compared with roughly 50 species that cause disease in humans (Agrios, 2005). As for insects, the first contact during plant-pathogen interactions happens on leaf surface except for some of them that are conveyed by insects, often by aphids. Many bacterial species are able to use wounds and natural openings, such as stomata and hydathodes, to go inside the leaf. Thanks to PAMPs perception and innate immunity, plants respond rapidly closing stomata, but many pathogens evolved effectors and toxins to force stomata opening or closing to get a humid environment suitable for their lifestyle (Melotto *et al.,* 2008). For pathogens using hyphal penetration to invade the host plant, the first barriers to overcome are cuticle and cell wall. OGAs generated during the penetration due to mechanical and chemical damages caused by the invader activate the PAMP-triggered immunity and the stomatal closure. Once inside the apoplast, it has to survive in a very hostile environment since apoplast is very rich in nutrients but also in chemicals that are the basis of innate immunity. Based on the invasion strategy and the mode of feeding, also pathogens can be distinguished in several groups: biotrophic, necrotrophic and hemi-biotrophic. The first group extracts nutrients from living cells infiltrating and establishing their *hyphae* within the cell. Necrotrophic fungi, instead, kill the cell before invasion taking nutrients that are released by damaged tissues. This life strategy is enabled by toxins and virulence factors that induces HR and cause massive cell death (Kliebenstein and Rowe, 2008). This latter strategy is difficult to overcome because the plant is impaired in its capacity to produce anti-microbial compounds. Hemi-biotrophic fungi combine both strategies during their life. At the beginning they assume a biotrophic lifestyle inserting their austoria within the cell, suppressing plant immunity and leading the spreading of the infection through the whole plant. Later, they assume a necrotrophic behaviour producing toxins and killing the cell. These different modes of invasion correspond to different response pathways involvement. Biotrophs mainly induce SA-mediated responses, while necrotrophs induce JA- and ET-signalling. The SA- and JA/ET-defence response pathways crosstalk and can act antagonistically, additively, or even synergistically depending on the intensity and duration of the signals provided to the host plant (Mur *et al.*, 2006). Furthermore, these pathways are interacting with other defence signals (ABA, auxin, GA, $H_2O_2$,

and NO) known to enhance or antagonize SA- and/or JA-defense signaling (Lopez *et al.,* 2008). This crosstalk can be manipulated by pathogens and herbivores to avoid effective host defences.

## 1.6 Phases of defence response

### *1.6.1 Insect elicitors*

Substances of biotic origin able to induce a defence response are defined "elicitors". Although herbivores and pathogens have different attacking strategy, they share the attitude to release molecules and patterns that can elicit plant responses. Elicitors can be produced by the pest but also by the attacked-plant. To distinguish the attack of herbivores from those from other biotic agents, plants are thought to have evolved the ability to perceive herbivory associated molecular patterns (HAMPs) (Felton and Tumlinson 2008; Mithofer and Boland 2008). Besides, the microbes located on insect surfaces can profit by the wounding and could also release elicitors. Finally, many insects harbor endosymbiotic microbes (Baumann, 2005) that are important in insect nutrition, but they could also be involved in elicitation of defence responses. HAMPs can be classified into two categories: (1) chemical elicitors derived from herbivore oral secretions (OS) and oviposition fluids; and (2) those that originate from the specific patterns of wounding (Wu and Baldwin, 2009). So, as for pathogen PAMPs, HAMPs could be highly conserved proteins, lipids or polymers necessary for insect life. These molecules could bind a receptor in plant activating the innate immunity. A wide group of elicitors contained in oral secretions are fatty acid-amino acid conjugates (FACs) that have been well characterized. Linolenic and linoleic acid are the core of these FACs, although oleic acid and other fatty acids are occasionally detected. All FACs are conjugated to glutamine or glutamic acid. Like PAMPs, FACs activate the SA-induced protein kinase, SIPK, and theWound-induced protein kinase, WIPK, in *N. attenuata*, which regulate ET, JA, and SA accumulation (Meldau *et al.,* 2009; Wu *et al.,* 2007). One of the firstly discovered FAC was volicitin, isolated from *Spodoptera exigua* OS. As reported by Turlings and collaborators (1993), when volicitin was applied to plant wounds the synthesis the release of a volatile blend similar to that induced by *S. exigua* feeding was observed. Interestingly,

volicitin lipidic part is produced by plants while the proteic component is produced by insects. Since then, FACs have been isolated from several lepidopteran species. Several FACs have been identified in *Manduca sexta* OS, the application of which to *Nicotiana attenuata*–wounded leaves induces the activation of MAPKs, JA, and ET biosynthesis and signaling, and the amplification and modification of wounding-induced transcriptomic, proteomic, and metabolomic responses, that have been shown to function as direct and indirect defenses in nature (Giri *et al.,* 2006). Furthermore, FACs were isolated also from crickets (*Teleogryllus taiwanemma*) and fruit flies (*Drosophila melanogaster*). FACs presence in several insect orders suggests its importance in insect physiology preventing the replacement strategy in order to overcome plant recognition and responses. Insect OS contain also proteic elicitors, such as β-glucosidase isolated from *Pieris brassicae* OS. Mattiacci and collaborators (1995) associated this molecule to terpenoids release in cabbage plants. The substrate of the insect β-glucosidase has not been identified but could be a glucose conjugated metabolite of plant or insect mid-gut origin. A glucose oxidase (GOX) was isolated from *Helicoverpa zea* OS and it appears that GOX suppresses plants' defence responses (Musser *et al.,* 2005). Inceptins are 11-residues peptides identified in *Spodoptera frugiperda*. When this insect feeds plant tissues, the ingested chloroplastic ATP synthase γ-subunit (cATPC) reacts with some enzymes in the insect midgut and it is converted in inceptin. Even a minute amount of inceptin introduced into mechanically damaged cowpea leaves dramatically augments the levels of JA, ET, and salicylic acid (SA) (Schmelz *et al.,* 2007). Recently, a new elicitor was discovered in American bird grasshoppers, caeliferin which is involved in the releasing of volatile terpenes from maize seedlings, as reported by Alborn *et al.,* 2007. Other eliciting components are released by labial and mandibular gland salivas. These are very relevant in responses to phloem-feeding insects. Hemipteran watery saliva has been widely characterized and is rich in Ca2þ-binding proteins, pectinesterases, PGs, lipases, peroxidases, phenoloxidases, amylases, cellulases, sucrases, proteases, and alkaline and acid phosphatases (Funk, 2001). Pectin-degrading enzymes may facilitate cell wall dissolution, stylet penetration, and release OGAs while proteases could facilitate digestion and nutrient assimilation. Saliva's composition is still under investigation. The species-specific composition is assessed, but Avr factors recognized by *R* gene products

have not been yet identified. Oviposition fluid also contains elicitors. Many herbivorous insects lay eggs on plants and some plants respond to oviposition by forming neoplasm and necrotic tissue, producing ovicidal substances and emitting volatile signals that attract parasitoids (Hilker and Meiners, 2006). In some cases, eggs injected into leaf tissue are isolated through the instauration of necrosis, elevated above the surface and may drop off. An example is the oviposition by Colorado potato beetle (*Leptinotarsa decemlineata*) results in a hypersensitive response–like necrosis in a potato plant, and the necrotic regions to which eggs are attached disintegrate and detach the eggs (Kruzmane *et al.,* 2002). Two compounds in the oviposition fluid were identified and associated to the elicitation of plant responses: bruchins and benzyl cyanide. The first one was isolated from the oviposition fluid of pea and cowpea weevils and consists in long chain diols that are mono- and diesterified with 3-hydroxypropanoic acid. In certain genotypes of peas, bruchins elicit neoplastic growth on pods, which lifts the egg out of the oviposition site and impedes larval entry into the pod (Kessler and Baldwin, 2002). The other compound, benzyl cyanide, was found in the oviposition fluid of large cabbage white butterfly (*Pieris brassicae*) and only 1 ng induces the arrest of parasitoid *Trichogramma brassicae* on Brussels sprout plants (*Brassica oleracea* var. *gemmifera* cv. Cyrus) (Fatouros *et al.,* 2008). A more insight in the elicitation events could improve the knowledge about early signalling and the transduction of responses in plant.

### *1.6.2 Pathogen elicitors*

PAMPs/MAMPs are highly conserved proteins, glycolipids and polysaccharides of microbes that contain motifs (or epitopes) that are perceived to activate innate immunity in plants and animals (Altenbach and Robatzek, 2007). As for insect, these molecules are necessary for pathogen life, so are difficult to mutate without compromising microorganism vitality. Therefore, PAMPs are the alarm through the plant perceives the presence of ‘‘non-self’’ molecules activating the PAMP-triggered immunity. After PAMPs recognition by LRR-RLKs, rapidly MAPK-signalling cascades and rapid influx $Ca^{2+}$ are activated. In addition to ROS production and emission of ET, all these events cause rapid changes in defence-related gene expression profiles and induce resistance to pathogens. Other

pattern-recognition receptors are receptor-like proteins (RLPs), which are LRR-RLKs lacking the cytosolic kinase domain (Altenbach and Robatzek, 2007). Bacterial PAMPs include flagellin, elongation factor-Tu, cold-shock protein, and lipopolysaccharide (LPS). Fungi are also rich sources of PAMPs, including cell wall- or plasma-membrane-derived molecules such as chitin and chitosan, L-1,3-glucan, heptaglucoside, xylanase, and ergosterol, as well as necrosis-inducing peptides (Nürnberger and Kemmerling, 2009). The best characterized PAMP is flagellin (flg22) which is recognized by FLS2 (Flagellin-sensing 2). After binding, FLS2 forms a complex with BAK1, that is a co-receptor of brassinosteroid receptor (BRI1), causing the phosphorilation and auto-activation of FLS2. In this way, FLS2-flg22 complex is fagocited inside the cell activating the PAMP-triggered signalling pathway. This process has a feedback control mediated by proteasome degradation of FLS2. As for insect, plants could also release OGAs during the interaction with the pathogen that are potent elicitors of defence responses against the necrotrophic fungus *Botrytis cinerea* (Ferrari *et al.,* 2007). Other PAMPs released during fungi infection are chitin (N-acetylchitooligosaccharides) and chitosan, in fact plants have pattern-recognition receptors that detect chitin fragments and chitosan (N-chitooligosaccharides) that have been characterized by Kaku research group (2006). These biopolymers are localized in fungal cell walls, nematode eggs and arthropod exoskeletons. Plants and microbes located on leaf surface release chitinases responsible of chitin fragmentation and elicitation of PAMP-triggered immunity.

### *1.6.3 Early signalling*

As for pathogens, plant responses against insects are induced after HAMPs recognition. Elicitors could be perceived on the cell surface and transduction of signal inside the cell by a second messenger. This hypothesis have been widely characterized for signalling in responses against pathogens. Receptors for pathogen elicitors have been identified and associated with the regulation of $Ca^{2+}$ influx and the activation of kinases. It's a long time that $Ca^{2+}$ have been identified as the second messenger involved in numerous signalling actions in all eukaryotes. $Ca^{2+}$ and other ions, such as $Na^{+}$ and $K^{+}$, influxes and the changes in their cytosolic concentrations are involved in stress responses and development

regulation. $Ca^{2+}$ influxes are differentially induced by different stress factors. The mechanical wounding-elicited $Ca^{2+}$ signal was weaker than that induced by herbivory, suggesting that an OS-recognition mechanism plays a role in activating $Ca^{2+}$ influxes (Maffei *et al.,* 2004). These regulation activities are mediated by plasmatic membrane depolarization and the following activation of various $Ca^{2+}$-responsive molecules like calmodulin, calmodulin-binding proteins, calcium-dependent protein kinases (CDPKs) and $Ca^{2+}$ binding proteins. Several CDPKs in Arabidopsis are involved in abscisic acid (ABA) signaling and thus plant resistance to drought or salt stress (Zhu *et al.,* 2007); besides, they have been associated to pathogen responses (Boudsocq *et al.,* 2010). Other important signal molecules are ROS, *Reactive Oxygen Species*, a class of molecules that include: the superoxide anion ($O^{2-}$), hydrogen peroxide ($H_2O_2$) and the hydroxyl radical ($^{\bullet}OH$). Their production is located on the external surface of plasmatic membrane, in peroxisomes, mitochondria and chloroplasts and the main step in their production is catalyzed by NADPH oxidase. Some evidence suggests that NADPH oxidases are the main source of wounding and herbivory-induced ROS in plants (Sagi *et al.,* 2004). A potato NADPH oxidase is phosphorylated by two CDPKs in a $Ca^{2+}$-dependent manner, which in turn elevates its ability to produce ROS (Kobayashi *et al.,* 2007). It's a long time that ROS have been associated to responses against pathogens and recent evidences support their implication also in herbivory-induced responses. In *Arabidopsis*, two *RBOH* genes (respiratory burst oxidase homologues, encoding an important subunit of NADPH oxidase), *AtrbohD* and *AtrbohF*, are required for pathogen-induced ROS production (Wu and Baldwin, 2010). In potato, the two subunits coded by these genes are directly phosphorylated by StCDPK4 and StCDPK5 enhancing the activity of NADPH oxidase and ROS release (Kobayashi *et al.,* 2007). In tomato, the antisense expression of the *RBOH* gene suppresses the expression of many defence genes, such as PIs (Sagi *et al.,* 2004). NO (Nitrogen Oxide) is one of the "active nitrogen species" and is a molecule indirectly involved in signalling and regulation of plant development, stomatal closure and stress responses. It's indirect involvement in defence is despatched by NO free radicals that allow the rapid modification of proteins involved in signal transduction. One of the most common protein modifications is S-nitrosylation that is associated to pathogen-induced hypersensitive response in Arabidopsis (Romero-Puertas *et al.* 2008). The

signalling cascade is then transferred to the wide and well-conserved among eukaryotes MAPK family proteins. MAP kinases and their regulatory phosphatases are widely used to integrate biotic and abiotic stress signals, as well as developmental cues. MAPK can be induced by salicylic acid (SIPK) and by wounding (WIPK) and both of them play a central role in plant responses to herbivory. SIPK and WIPK in potato are involved in the Mi-mediated resistance to aphids (Li *et al.,* 2006). Besides, LeMPK1/2/3 had been shown to function in host-specific AvrPto-dependent resistance to the bacterial pathogen *P. syringae* (Pedley *et al.,* 2004). All MAP kinase cascades utilize a hierarchy of MAP3Ks (MAP2K kinases or MEKKs), MAP2Ks (MAPK kinases or MKKs), and MAPKs (MAP kinases or MPKs). In tomato, Kandoth and collaborators (2007) using a VIGS (Virus Induced Gene Silencing) strategy observed that LeMPK1 and LeMPK2 are required for systemin-mediated resistance to *M. sexta* larvae revealing that these are essential signalling components not only of pathogen-induced defences but also in confering resistance to herbivores. So from this studies, the activation of specific components of MAP signalling cascade could direct responses against different damaging agents interesting different defence pathways. While, as mentioned before, MPK1 and MPK2 mediate responses against wounding and herbivores, MPK3 and MPK6 have been associated to the regulation of ACS6 (1-amino cyclopropane-1-carboxylic acid synthase 6) which catalyzes an important step in ethylene biosynthesis. Besides, these kinases activate PR genes and SAR through the regulation of the WRKY 22/29 transcription factors. MPK4, instead, have been associated to JA- and ET-mediated responses to necrotrophic fungi through the involvement of other transcription factors of WRKY family. Nuclear-localized MPK4 is a negative regulator of SA-regulated defenses and promotes ET- and JA-mediated defences and resistance to necrotrophs (Brodersen *et al*., 2006). There are three additional MKK cascades (MKK3, MKK7, and MKK9) that contribute to *Arabidopsis* defence against pathogens (Dóczi *et al.,* 2007).

### *1.6.4 Response effector phase: hormone-regulated pathways crosstalk*

The gene expression changes that occur within 30 min to 1 h after flg22, chitin, and OGAs treatments overlap extensively with each other (Denoux *et al.,* 2008).

The faster induced genes are those coding for signalling components, such as kinases and receptors, and regulating components like transcription factors that subsequently regulate late defence genes expression. After 2 h many defense genes coding for enzymes involved in phenylpropanoid, JA-, ET- and SA-mediated pathways are induced pushing the activation of these responses. Late defence genes, such as PPO, Lap, PIs, peroxidases and PR genes, are rapidly activated manifesting their anti-nutritional and anti-microbial effects. Besides, genes involved in the promotion of indirect defences are also activated. The activation of defence responses is controlled by different hormone-regulated pathways that often overlap. Plants produce a wide variety of hormones, which include auxins, gibberellins (GA), abscisic acid (ABA), cytokinins (CK), salicylic acid (SA), ethylene (ET), jasmonates (JA), brassinosteroids (BR) and peptide hormones. Plant hormones play important roles in diverse growth and developmental processes as well as various biotic and abiotic stress responses in plants (Bari and Jones, 2009). SA is generally associated to responses against biotrophic and hemi-biotrophic pathogens as well as the establishment of systemic acquired resistance (SAR). JA and ET are usually associated to responses against necrotrophic pathogens and herbivores. As mentioned before, these hormone-regulated pathways crosstalk determining an antagonistic or synergistic interaction depending on timing and dosage of released hormones. In addition, the lifestyles of different pathogens are not often readily classifiable as purely biotrophic or necrotrophic. Therefore, the positive or negative cross talk between SA and JA/ET pathways may be regulated depending on the specific pathogen (Adie *et al.,* 2007). A wide knowledge about responses mediated by these hormones has been implemented using mutants impaired in defence responses. In order to investigate on JA-signalling three main mutants were produced: *Arabidopsis coi1* (*coronatine insensitive 1*) that is insensitive to JA; *jar1* (*Jasmonate resistant 1*) and *jin1/myc2* (*jasmonate insensitive 1*). COI1 encodes an F-box protein involved in the SCF-mediated protein degradation by the 26S proteasome and is required for most JA-mediated responses (Xie *et al.,* 1998). JAR1 encodes a JA amino acid synthetase involved in the conjugation of isoleucine to JA (JA-Ile) which is considered to be the bioactive JA molecule perceived by plants (Staswick and Tiryaki, 2004; Thines *et al.,* 2007). JIN1/MYC2 encodes a transcription factor involved in the transcriptional regulation of some JA

responsive gene expression (Lorenzo *et al*., 2004). Thines and collaborators (2007) discovered JAZ proteins (JA-ZIM domain protein) that interact with MYC2 transcription factor interfering with the expression of JA-controlled defence genes. Jasmonate promotes interaction between JAZ proteins and the SCFCOI1 ubiquitin ligase, leading to JAZ degradation via the 26S proteasome in *Arabidopsis thaliana* allowing MYC2 to activate defence genes (Sun *et al.,* 2011). Several studies indicate that JA- and ET work cooperatively inducing the expression of pathogen-related defence genes. Although it is a very small molecule, ET regulates a wide range of physiological processes linked to plant development and stress response. The positive relationship with JA-signalling has been underlined by Lorenzo and collaborators (2003) by the identification of an Arabidopsis transcription factor, ethylene response factor 1 (ERF1), that positively regulates JA- and ET-responsive genes. ET biosynthesis is induced by herbivore attack and by *M. sexta* OS in *Nicotiana attenuata* (von Dahl *et al.,* 2007). The Arabidopsis transcription factor MYC2 has also been shown to regulate the interaction between JA and ET mediated defence signaling. ACS (1-amino cyclopropane synthase) and ACO (1-amino cyclopropane oxidase) genes, that code for key enzymes involved in ethylene biosynthesis, are regulated transcriptionally after herbivory elicitation. As mentioned above, Joo and collaborators (2008) established MPK6 involvement in ET biosynthesis by phosphorilation of ACS and ACO. One of the important regulatory components of SA signalling is non-expressor of PR genes 1 (NPR1), which interacts with TGA transcription factors that are involved in the activation of SA-responsive PR genes (Dong, 2004). *Arabidopsis npr1* plants are compromised in the SA-mediated suppression of JA responsive gene expression indicating that NPR1 plays an important role in SA-JA interaction (Spoel *et al*., 2007). WRKY and MAPK are important communicators between SA and JA. WRKY70 acts as a positive regulator of SA-dependent defences and as a negative regulator of JA-dependent defences playing a central role in determining the balance between these two pathways. Recently, WRKY62 has been reported to be induced by MeJA and SA synergistically (Bari and Jones, 2009). Auxins are another class of plant hormones promoting the degradation of transcriptional repressors called (Aux/IAA). Auxin induce the expression of three groups of genes: Aux/IAA family, GH3 family and small auxin-up RNA (SAUR) family. Recently, it has been shown that GH3.5 acts as a bifunctional modulator in both SA and auxin signaling during

pathogen infection (Zhang *et al.,* 2008). Besides, the over-expression of GH3-8 enhanced rice resistance to *Xanthomonas oryzae pv. oryzae (Xoo)* with the contemporary reduction of SA and JA level, suggesting a SA and JA independent resistance (Ding *et al.,* 2008). Recent findings about auxin interaction with other defence pathways associated this hormone to the attenuation of plant defence responses (Bari and Jones, 2009). Arabidopsis mutants impaired in auxin transport and signalling were more susceptible to the necrotrophic fungus *Botrytis cinerea* and *Plectosphaerella cucumerina* (Llorente *et al.,* 2008). This pathway have been also related to virus defence by Padmanabhan and collaborators (2008). They established this relationship through the observation of the interaction of tobacco mosaic virus (TMV) replicase with Aux/IAA proteins affecting the expression of auxin-responsive genes. ABA is another important plant hormone involved not only in growth and development, but also in plant defence showing varied activities. ABA is shown to be involved in the negative regulation of plant defence against various biotrophic and necrotrophic pathogens. For example, the ABA-deficient *sitiens* mutant of tomato showed more resistance to *B. cinerea* (Asselbergh *et al.,* 2001). ABA also showed a positive regulation of defence responses. In fact, it activates stomatal closure interfering with pathogens invasion. This assumption is confirmed time ago by the study of Whenham and collaborators (1982) in which tobacco plants infected with TMV resulted in increased ABA levels. Reversely, ABA treatment on tobacco plants enhanced resistance to TMV. Moreover, Adie and collaborators (2007) also demonstrated that ABA is required for JA biosynthesis and the expression of JA responsive genes after *P. irregulare* infection. Besides, ABA has been shown to induce resistance partly through priming the deposition of callose (Flors *et al.,* 2008). ABA is also involved in ROS intermediates production regulating defence responses not only against biotic agents but also abiotic stress responses. Brassinosteroids are another class of plant hormones and is the unique sharing similarities with animal hormone. They influence many physiological processes plus responses to environmental stresses. Recently, it has been reported that BR enhances resistance to TMV and *Oidium* sp. in tobacco (Bari and Jones, 2009). BRI1 associated kinase (BAK1) interacts with BR receptor (BRI1) inducing basal defence and programmed cell death (Chinchilla *et al.,* 2007). As mentioned before, BAK1 interacts with FLS2, the flagelling receptor, participating to the PAMP-

triggered immunity. BR have also been associated to the biosynthesis of other hormones, such as JA and ET since the regulation of two key enzymes involved in their synthesis, respectively, OPR3 and ACC synthase. The regulation of defence responses is also controlled by peptide hormones: systemin and hydroxyproline-rich systemin glycopeptides from tomato (Narvaez-Vasquez *et al.,* 2007) and AtPep from Arabidopsis (Huffacker *et al.,* 2006). These peptides are from 18 to 23 amino acids in length, are processed from wound- and JA-inducible precursor proteins, and play roles in the activation of local and systemic responses against wounding and pest attack. Plant hormone signaling pathways are not isolated but rather interconnected with a complex regulatory network involving various defence signalling pathways and developmental processes (Bari and Jones, 2009).

### 1.7 Systemin- and Jasmonate-mediated signalling

Green and Ryan (1972), in their pioneer study about tomato wound-inducible proteinase inhibitors, discovered a small plant hormone induced by wounding and herbivory, called systemin (Sys). This is a 18-amino acids oligopeptide located in the cytosol released by a larger precursor protein of 200-amino acids called prosystemin (ProSys). In tomato genome there is a single copy of *ProSys* gene containing 11 exon of which the last one codes for systemin. ProSys homologs have only been found in species of the Solaneae subtribe of the Solanaceae family, including tomato, potato, bell pepper, and nightshade, but it is not found in tobacco or *Arabidopsis* (Constabel *et al.*, 1998). *ProSys* gene is under tissue, developmental and environmental control. It is induced by wounding, chewing insects, JA application and pathogen OGA. Its over-expression under the regulation of the CaMV 35S promoter in transgenic tomato plants led to the constitutive accumulation of high levels of several defensive proteins in leaves (McGurl *et al.,* 1994). Using the opposite approach, McGurl and collaborators (1992) demonstrated that transgenic tomato plants impaired in the accumulation of PIs due to the suppression of *ProSys* expression, using an antisense strategy, maintained a robust local wound response, probably due to OGAs and other elicitors release able to start JA-signalling. In fact, JA and MeJA (Methyl Jasmonate) applications on tomato plants has been shown to induce the synthesis of defensive PIs not only in the treated plants but also in the neighbours (Farmer

and Ryan, 1990). Ryan research group widely studied ProSys and its signalling pathway. In 1999 they identified a putative Sys receptor, SR-160, that was a serine/threonine receptor kinases with extracellular LRR domain (leucine-rich repeats), which was found to be highly similar to the *Arabidopsis* brassinolide receptor BRI1. SR-160 function was investigated through its expression in tobacco by Scheer (2003). In transgenic tobacco expressing *SR-160* mRNA constitutively, the Sys receptor is membrane localized, binds Sys and activates early defense responses (Scheer *et al.*, 2003). After binding its receptor, Sys starts the depolarization of plasma membrane, alkalinization of apoplast, $Ca^{2+}$ influxes and $H_2O_2$ release. These events activate MAPK signalling and phospholipase A2 (PLA2) that tears out α-linolenic acid (LA) from membrane starting the octadecanoid pathway in which JAs (12-OPDA, JA, MeJA, JA-Ile) are produced. After a series of reactions catalyzed by enzymes localized in chloroplasts [lipoxygenase (LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC)], LA is further converted to 12-oxo-phytodienoic acid (OPDA). After its transport to peroxisomes, OPDA reacts with OPDA reductase (OPR); after three steps of β-oxidation, JA is formed. A ramification of this pathway produces GLVs, "green leaf volatiles". JA-Ile is the JA derivative provided of biological activity. Schmiller and Howe (2005) proposed a model for systemin-signalling pathway showed in fig. 2.

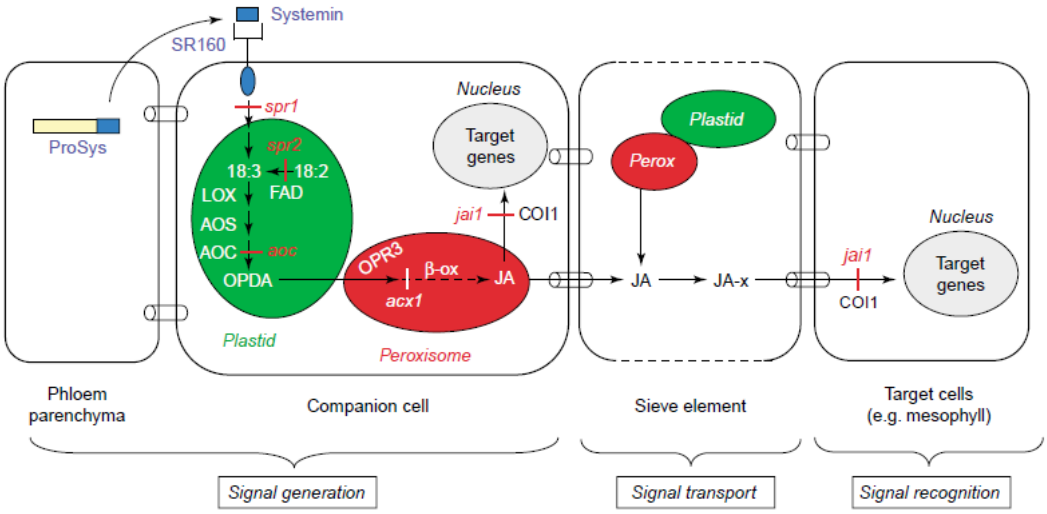


**Figure 2. Systemin-mediated signal transduction pathway. Image from Schmiller and Howe (2005).**

Interestingly, this signalling pathway interests different compartments of the plant: it starts in the phloem reaching the nucleus of target cells not only in the damaged site, but also distally. To shed more light on systemin role in the development of defence responses and the relationship with JA in the mediation of a systemic signal, several mutants defecting in receptor (Li *et al.,* 2004). JA relevance in Sys-signalling pathway was demonstrated through systemin-signalling were produced. Tomato *spr1* are impaired in systemin perception and development of a systemic response (Lee and Howe, 2003).This is due to the failure accumulation of JA in response to exogenous application of systemin. Tomato *spr6* mutants are impaired in systemin- and wound-induced responses. spr6 is allelic to jai1-1, which is a loss-of-function allele of the tomato homolog of CORONATINE-INSENSITIVE1 (COI1), an F-box protein that has been shown to be the jasmonate *spr6* mutation in *35S:prosystemin* transgenic plants that caused Sys inability in the induction of PPO (polyphenol oxidase) and PIs. Tomato *spr2* mutant produces a loss-of-function fatty acid desaturase and is impaired in wound induced JA biosynthesis and the production of a long-distance signal for the expression of defensive PI genes. A recent investigation carried out by Li and collaborators (2002) about Sys and JA involvements in plant systemic response underlined the needing of JA synthesis for the defence signal transmission. Using *spr2* and *jai-1* mutants, they suggested that JA or someone JA derivative may act as a mobile signal through the plant. As mentioned above, s*pr1* mutants are impaired in systemic response, but not in the local one. In fact, they showed normal PIs induction after the induction of local wound response by OGA application. PIs induction was not observed in distal undamaged leaves. In this study the failure transmission of the long-distance signal in s*pr1* mutants was attributed to the impairment in octadecanoid pathway induction in undamaged leaves. The systemin-specific character of spr1 raises the possibility that the Spr1 protein might be a systemin specific component that directs the systemin signal flow into a pathway leading to JA biosynthesis and *PI* gene activation (Sun *et al.,* 2011).

Recently, Narvaez-Vasquez and collaborators (2007) added to the systemin-signalling pathway new molecules discovered firstly in tobacco and later in tomato, the hydroxyproline-rich systemin (ProHypSys). As systemin, they are cleaved by a larger precursor protein but are not localized in the cytoplasm since they have a signal peptide for the cell-wall localization. The isolated peptide from tobacco and

tomato were able to induce medium alkalinization and MAPK activation. In both tobacco and tomato, the *Pro-HypSys* genes are upregulated in the leaves by wounding, Sys, MeJA, and the HypSys peptides themselves (Pearce *et al.,* 2001; Pearce and Ryan, 2003). Their over-expression in tobacco and tomato caused the constitutive up-regulation of defence genes as determined by ProSys over-expression and an enhanced resistance to insects. In addition, downregulation of *TomHypSys* gene expression by antisense technology also diminished the systemic wound inducibility of defense genes, suggesting that Sys and HypSys work cooperatively to upregulate the systemic wounddefense response in tomato (Narvaez-Vasquez *et al.,* 2007). Recently, Huffacker (2006) isolated and characterized similar peptides in *Arabidopsis,* called AtPep that are activated by wounding, MeJA and ET. Also these 23-aa peptides are cleaved by a larger precursor protein (ProAtPep), bind LRR receptors isolated by Yamaguchi and collaborators (2010) inducing defence genes. The gene encoding ProAtPep1 is part of a small family of at least seven members in *Arabidopsis*, with orthologs in species of many other families, including important crop plants (Schaller, 2008). This gene is expressed constitutively in all tissues and is induced by wounding, MeJA and ET. These peptides induce defensin (*PDF1.2*) and $H_2O_2$ release activating plant innate immunity. There is no sequence homology between the protein precursors of Sys, HypSys, and AtPep1 peptides. The systemin family is present only in *Solanaceae* despite wound induced systemic response is shared by all plant kingdom. All these peptides show sequences rich in proline, hydroxyproline and charged residues with common motives that advance the hypothesis of a common origin. Proline residues are known to confer structural conformations in the backbone chains of bioactive peptides that are important for the interactions of peptide ligands with their receptors (Rath *et al.*, 2005). The evolution of these peptides and their interaction with development are varied. JA signalling regulates development and defence responses in the plant kingdom and have been widely studied in model species like tomato and *Arabidopsis*, finding many differences. For example, *Arabidopsis* misses wound-induced proteinase inhibitor and polyphenol oxidase genes. By comparison between tomato and *Arabidopsis* mutants impaired in JA-signalling or production, a major JA impact on development was observed in *Arabidopsis* since the percentage of male sterility was very high. A common feature are JAMYC transcription factors coding for

protein able to bind promoters of JA-induced defence genes. In fact, these transcription factors are conserved among many plant species. Another common element between tomato and Arabidopsis in JA-signalling pathway are JAZ family proteins, repressor of JAMYC transcription factors.

### 1.8 Research objectives

The aim of this research activity is to shed more light on ProSys involvement in tomato responses against several stress condition and to evaluate of a possible use of this plant hormone as a broad-spectrum defence instrument. In particular, *ProSys* involvement in signal transduction of responses to wounding and chewing insects have been widely studied, while the effect on phloem-feeders and pathogenic fungi have not been yet characterized. As reported by Oerke and collaborators (2004) these are serious damaging pests that cause a lot of losses to the main cash crops. These pests reduce production yield and cause reductions in its economical value. Since ProSys is involved in the regulation of defence signalling in tomato and since it induces the production of volatile compounds attracting the parasitoid *Aphidius ervi* that attack several aphid species (Corrado *et al.,* 2007), its direct link with aphid responses was investigated. For this reason, tomato cv. “Red Setter” was choosen to over-express *ProSys* gene since the lacking of the dominant allel at *Mi* locus, a gene involved in resistance against aphid and nematodes (Rossi *et al.,* 1998). In this way, a putative resistance of transgenic plant to the aphid *Macrosiphum euphorbiae* could be specifically attributed to *ProSys* over-expression. Another purpose is to investigate about ProSys involvement in responses to pathogenic fungi. For this reason, bioassay and time course gene expression analysis after inoculums of the necrotrophic fungus *Botrytis cinerea* were carried out in order not only to evaluate resistance/susceptibility of transgenic plants, but also to identify key genes involved in this process. In order to shed more light on ProSys involvement and cooperation with JA in the transmission of the systemic signal, time-course analysis of *ProSys* and *wound-induced proteinase inhibitor I (InhI)* expressions was carried out after *Spodoptera littoralis* feeding. In order to get a global vision on transcriptomic profiles determined by *ProSys* over-expression, a microarray approach was used. The functional analysis of differentially expressed genes had

the aim to underline defence-related functions and involvement in pests-induced pathways. For a deeper and completer analysis, all differentially expressed sequences were translated in their nearest homologous Arabidopsis protein in order to carry out a prediction study of protein-protein interactions. This approach helped to observe the direct link between ProSys-regulated main players of responses to varied stress conditions.

# 2. MATERIALS AND METHODS

## 2.1 Transgenic vectors and expression cassettes

The recombinant binary vector used for tomato genetic transformation, deriving from pKYLX is described in Rocco *et al* (2008). The pMZ plasmid (Fig. 3) contains an expression cassette carrying the full length *prosystemin* cDNA (acc. num. M84801) under the control of the constitutive promoter CaMV $35S^2$ and RbcS terminator.

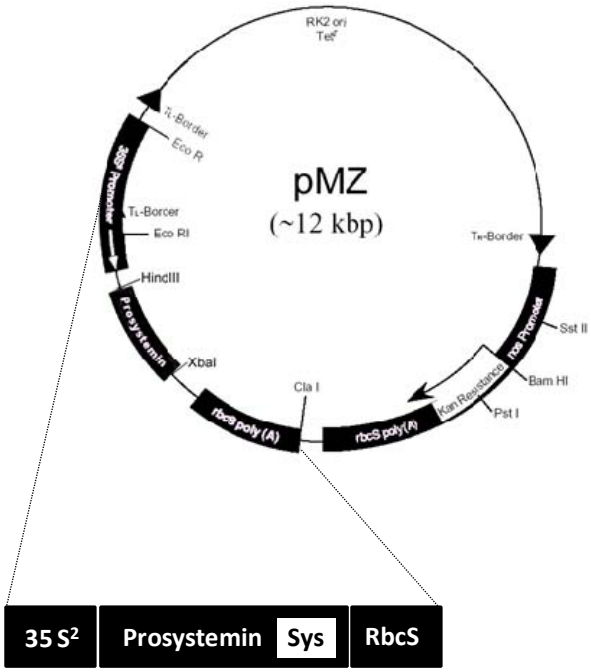


**Figure 3. The image displays the pMZ plasmid that contains the full length prosystemin cDNA (Rocco *et al.,* 2008).**

### *2.1.1 DNA plasmid isolation and quantification*

A single colony of *A. tumefaciens* LBA4404 cells containing the transgenic vector was inoculated in 3 mL of YEP medium (10 g/L yeast extract, 10 g/L peptone, 5 g/L NaCl; Oxoid) supplemented with 100 mg/L rifampicin, 500 mg/L streptomycin and 50 mg/L Kanamycin and were incubated at 28°C for 16 hours shacking at 180 rpm. Plasmid DNA isolation was carried out as described (Birnboim and Doly, 1979) following an alkaline lysis procedure which consists in the use of 1.5 mL of bacterial culture and centrifugation at 14000 rpm at 4°C for 30 sec, pellet suspension in 100 µL of Sol I (50 mM glucose, 25 mM Tris-Cl pH 8.0, 10 mM

EDTA pH 8.0) followed by vigorous shaking and incubation on ice for 5 min. The following step consisted in the addition of 200 µL of Sol II (0.2 M NaOH and 1% SDS) and shaking by tubes inversion, incubation on ice for 5 min and then the addition of 150 µL of Sol III (60% 5M potassium acetate and 11.5% acetic acid) followed by shaking and centrifugation at 14000 rpm at 4°C for 15 min. Supernatant was transferred in a new eppendorf tube, added with 0.1 vol of 3M sodium acetate pH 5.2 and 2 vol of ethanol followed by incubation at -80°C for 5 min, centrifugation at 14000 rpm at 4°C for 15 min. Finally pellet was washed with 70% ethanol and suspended in sterile water. DNA was quantified through agarose gel electrophoresis by comparing with known quantities of Bacteriophage Lambda DNA (Promega). 2 µL of samples were added with 1X loading buffer (50% glycerol, 0.25% w/v bromophenol blue, 0.25% w/v xylene cyanol; Sigma). Samples were loaded on a 0.8% w/v agarose gel prepared with the addition of ethidium bromide (1:40000 diluted) in 1X TAE buffer (40 mM Tris-Acetate, 1mM EDTA) (Sambrook *et al*., 1989) and a 80V potential difference was applied for 30 min. DNA bands were visualized using UV light (UV Gel Doc BIORAD) and samples concentration was estimated comparing their fluorescence with λ DNA bands.

### *2.1.2 PCR (Polymerase Chain Reaction)*

Twenty ng of isolated plasmidic DNA were used in the PCR reaction that was prepared in a final volume of 20 µL using 1X Green GoTaq buffer (Promega) that is ready to be loaded on the agarose gel, 0.2 mM dNTPs (Promega), 0.5 µM of each primer (BBSBB Fw and RbcS Rv; Table 1) and 1 U GoTaq (Promega). PCR conditions were the following: 94°C for 5 min, 30 cycles at 94°C for 30 sec, 53°C for 30 sec, 72°C for 30 sec and a final polymerization at 72°C for 7 min. The whole reaction volume was loaded on a 2% (w/v) agarose gel performing electrophoresis as described before. Products size was evaluated through comparison with a size marker, 1 Kb Plus Ladder (Invitrogen).

## 2.2 CAPS analysis

The analysis of cleaved amplified polymorphic sequence (CAPS) of the REX-1 marker associated to *Mi* resistance genes (acc. num. AF039682) was carried out on *Solanum lycopersicum* cv. “Red Setter” using cv. “Motelle” DNA as positive control. DNA was isolated from tomato leaves as described (Fulton *et al.,*1995) starting from 2 leaf discs powdered in liquid nitrogen. 750 µL of Microprep buffer, composed by 2.5 parts of DNA Extraction Buffer (0.35M D-Sorbitol, 0.1 M Tris base, 5 mM EDTA, pH 7.5), 2.5 parts of Nuclei Lysis Buffer (0.2 M Tris-HCl, 0.05 M EDTA, 2 M NaCl, 2% w/v CTAB), 5% w/v Sarkosyl and 0.3 g Sodium Bisulfide, were added to each eppendorf tube containing the leaf powder and were incubated at 65°C from 30 min to 2 h. Tubes were then filled with chlorophorm and centrifuged at 13000 rpm at RT for 5 min. Upper phase was transferred to new eppendorf tubes and DNA was precipitated with 1 volume of cold isopropanol and centrifugation at 13000 rpm at 4°C for 5 min. Supernatant was discarded, pellet dried and washed with 70% ethanol and finally suspended in 50 µL of sterile water. After quantification (par. 2.1.1), 100 ng of isolated DNA were used in the PCR reaction that was prepared in a final volume of 50 µL using 1X GoTaq buffer (Promega), 0.2 mM dNTPs (Promega), 0.5 µM of each primer (Mi REX-1 Fw and Mi REX-1 Rv; Table 1) and 1 U GoTaq (Promega). PCR conditions were the following: 94°C for 5 min, 30 cycles at 94°C for 45 sec, 55°C for 45 sec, 72°C for 1 min and a final polymerization at 72°C for 7 min. 20 µL of PCR products were added with 1X loading buffer (par. 2.1.1) and loaded on a 1.5% w/v agarose gel as amplification control. 17.5 µL of the remainder were added with 1X Buffer E and 0.5 U *Taq*I (Promega) and incubated at 65°C for 3 h. Digestion products were checked by electrophoresis as described in par. 2.1.2.

**Table 2. Primer sequences, amplicon length, gene symbols and accession number of genes specific primers used for the expression analysis.**

| Primer | Sequence (5'→3') | Amplicon length (bp) | Gene Symbol or name | Accession Number (Reference) |
|---|---|---|---|---|
| EF Fw Rt | CTCCATTGGGTCGTTTTGCT | 101 | *EF1-α* | X53043 (Shewmaker *et al.*1990) |
| EF Rv Rt | GGTCACCTTGGCACCAGTTG | | | |
| BBSBB Fw | GGGAGGGTGCACTAGAAATA | 110[1] 717[2] | *Prosys* | M84801.1 M84800.1 (McGurl *et al.* 1992) |
| BBSBB Rv | TTGCATTTTGGGAGGATCAC | | | |
| BBSBB Fw | GGGAGGGTGCACTAGAAATA | 161 | *ProSys* | M21375 (Hunt *et al.,* 1988) |
| RbcS Rv | TTGTCGAAACCGATGATACG | | | |
| TomInhI Fw | GAAACTCTCATGGCACGAAAAG | 114 | *InhI* | K03290 (Ryan C.A. *et al.* 1985) |
| TomInhI Rv | CACCAATAAGTTCTGGCCACAT | | | |
| TomInhII Fw | CCAAAAAGGCCAAATGCTTG | 116 | *InhII* | K03291 (Ryan C.A. *et al.* 1985) |
| TomInhII Rv | TGTGCAACACGTGGTACATCC | | | |
| TomMCPI Fw | CACAAGACGATTGTTCTGGTGG | 106 | *Mcpi* | X59282 (Martineau B. *et al.,* 1991) |
| TomMCPI Rv | TTGTAATCACACGCCTATGGCC | | | |
| Mi REX1 Fw | TCGGAGCCTTGGTCTGAATT | 650 | *Mi* | AY949616 (Williamson *et al.,* 1994) |
| Mi REX1 Rv | GCCAGAGATGATTCGTGAGA | | | |
| StbEF Fw | AAGCTGCTGAGATGAACAAG | 687[3] 767[4] | *EF1-*α | X14449.1 X53043.1 (Shewmaker *et al.*1990) |
| LeEF Rv | GTCAAACCAGTAGGGCCAAA | | | |
| PR1a Fw | ATGCAACACTCTGGTGGACCTT | 101 | *PR1a* | EU589238 (Chen *et al.,* 2007) |
| PR1a Rv | CCATTGCTTCTCATCAACCCA | | | |
| Arginase Fw | AGGTCTCTCTTTCCGCGATGT | 104 | *ARG1* | AY656837 (Chen *et al.*2007) |
| Arginase Rv | CATCAACAGTATCACGCTGCG | | | |
| JA-ZIM Fw | AATCGCGAGACGGAATTCACT | 135 | *JAZ1* | AK327683 (Aoki *et al.,* 2010) |
| JA-ZIM Rv | GCACCTAATCCCAACCATGCT | | | |
| Kunitz Fw | TTGTTGGAGACGGAAGGAAGC | 128 | *Kunitz-type proteinase inhibitor* | X73986 (Werner *et al.,* 1993) |
| Kunitz Rv | CGGCAAAATGGACAAAGCAC | | | |
| LoxD Fw | TTCATGGCCGTGGTTGACA | 101 | *LoxD* | SLU37840 (Hetiz *et al.,* 1997) |
| LoxD Rv | AACAATCTCTGCATCTCCGG | | | |
| Mate Fw | ACCCATCAATGACACCCAAG | 154 | *Mate efflux protein* | BI933305 |
| Mate Rv | GGCATGTGGTATGGGATGTT | | | |
| PPO Fw | TTTGATAGGCGGAGTTTGCG | 111 | *PPO* | BI925947 (Van der Hoeven *et al.,* 2002) |
| PPO Rv | CCACCAGTTCAGTTATCGCCA | | | |
| PR10 Fw | CATCATGTGACCACGAATGGA | 101 | *PR10* | AK329477 (Aoki *et al.,* 2010) |
| PR10 Rv | AACGTGAAGGACAAAACCCAAG | | | |
| PtoRespLoc Fw | TTGCTATGGCTCGTAGAGCAAC | 143 | *Pto* | TC223474 |
| PtoRespLoc Rv | CGACGCCGTTTCTTCTTCTT | | | |
| SAM Fw | GCCAAAAATTCTCTGCTTCAGC | 128 | *SAM* | ES894405 (Besser *et al.,* 2007) |
| SAM Rv | GAACAACCTAAATCCGCAATGC | | | |
| TrDeam Fw | TTAGACGCTTTCTCCCCTCGT | 101 | *Td* | M61914 (Samach *et al.,* 1991) |
| TrDeam Rv | GCTTGAGGAACTTGGAATCCC | | | |

[1] Product obtained by cDNA amplification
[2] Product obtained by genomic DNA amplification

[3] Product obtained by cDNA amplification
[4] Product obtained by genomic DNA amplification

| | | | | |
|---|---|---|---|---|
| Subtil Fw<br>Subtil Rv | TCAATGGCTGCTCCTCACATT<br>TGCAGTGGTCATCATGGCA | 102 | *Sbt* | TA38526_4081<br>(Jorda *et al.,* 1999) |
| Pti Fw<br>Pti Rv | TTCGCGATTCGGCTAGACAT<br>GCCTTAGCACCTCGCATTCTAA | 111 | *Pti5* | U89256<br>(Asselbergh *et al.,* 2001) |
| LoxA Fw<br>LoxA Rv | ATACACATGCTGTGATCGAGCC<br>TGTGTCCCGGAAATGAGGAT | 100 | *LoxA* | SGN-U143303<br>(Asselbergh *et al.,* 2001) |
| Mir Fw<br>Mir Rv | GATACTCCGTACAGGCGTCGAT<br>TGGACAACAGCATCAAGTGGA | 113 | *LeMir* | SGN-U144553<br>(Asselbergh *et al.,* 2001) |
| Ext Fw<br>Ext Rv | CTTGGCCTTTTTTTGGCCAT<br>CGCCATATTCGGCTTCATGT | 141 | *Ext1* | X55688<br>(Asselbergh *et al.,* 2001) |
| Arg Fw<br>Arg Rv | CGTTCCGCGATGTTCTAAACAT<br>TCGCAGCAACCATTGCAGT | 119 | *ARG2* | AK321112<br>(Asselbergh *et al.,* 2001) |
| LapA Fw<br>Lap A Rv | ATCTCAGGTTTCCTGGTGGAAGGA<br>AGTTGCTATGGCAGAGGCAGAG | 99 | *LapA2* | U50152<br>(Gu *et al.,* 1996) |
| Osmotin Fw<br>Osmotin Rv | CCAATATAAACGGTGAATGCCC<br>GACCACATGGACCGTGATTACA | 110 | *Osmotin 34* | AK322591<br>(Asselbergh *et al.,* 2001) |
| WRKY Fw<br>WRKY Rv | GAAAGACAGGCAGCCACTAGGA<br>GCCCATCCCATTTTCACGT | 103 | *WRKY40* | AK326455<br>Rodriguez-Saona *et al.,* 2010) |

## 2.3 Tomato genetic transformation

### *2.3.1 Media composition*

Tomato genetic transformation was performed as described (van Roekel et al., 1993) with a different co-cultivation time and without using a feeder layer. Media used are shown in table 3 and were prepared according to Duchefa Catalogue (2010-2012).

**Table 3. Media composition for tomato genetic transformation.**

| | MS 30 | TRI 1 | GCF 10 | GCF 11 | TRI 2 |
|---|---|---|---|---|---|
| **Sali MS** | 4.3 g/L | 2.2 g/L | 4.3 g/L | 4.3 g/L | 2.2 g/L |
| **Tiamina** | 0.4 mg/L | 0.2 mg/L | 0.4 mg/L | 0.5 mg/L | 0.2 mg/L |
| **Myo-Inositolo** | 100 mg/L | 50 mg/L | 100 mg/L | 100 mg/L | 50 mg/L |
| **Glicina** | - | - | - | 2 mg/L | - |
| **Piridossina** | - | - | 0.5 mg/L | 0.5 mg/L | - |
| **Acido folico** | - | - | - | 0.5 mg/L | - |
| **Biotina** | - | - | - | 0.05 mg/L | - |
| **Zeatina riboside** | - | - | 1.5 mg/L | 1.9 mg/L | - |
| **NAA** | - | - | - | - | 0.1 mg/L |
| **IAA** | - | 0.2 mg/L | 0.2 mg/L | - | - |
| **Ancymidol** | - | - | - | - | 0.5 mg/L |
| **Acido nicotinico** | - | - | 0.5 mg/L | 4.9 mg/L | - |
| **Saccarosio** | 30 g/L | 15 g/L | 30 g/L | 30 g/L | 15 g/L |
| **Carbenicillina** | - | - | 500 mg/L | 500 mg/L | 250 mg/L |
| **Kanamicina** | - | - | 50±10 mg/L | 50±10 mg/L | 25 mg/L |
| **Agar** | - | 8 g/L | 8 g/L | 8 g/L | 8 g/L |
| **pH** | 5.8 | 5.9 | 5.9 | 5.9 | 5.9 |

### *2.3.2 Seeds germination and sterilization*

*Solanum lycopersicum* cv. “Red Setter“ seeds were sterilized in 70% ethanol for 5 minutes, moved to fresh 70% ethanol for 1 minute and then sterilized in 10% bleach and 0.1% SDS for 10 minutes and washed in sterile water for 5 times. Sterile seeds were put to germinate on TRI1 medium with a photoperiod of 16:8 hr light/dark under 3000 lux light intensity. Cotyledons were used when completely expanded before appearing of “real leaves”.

*2.3.3 Agrobacterium tumefaciens culture preparation*

A single colony of *A. tumefaciens* LBA4404 cells containing the transgenic vector was inoculated in 10 mL of AB medium (60 g/L $K_2HPO_4$, 20 g/L $NaH_2PO_4$, 20 g/L $NH_4Cl$, 3 g/L KCl, 5 g/L Glucose, 6 g/L $MgSO_4$ 7H2O, 0.2 g/L $CaCl_2$, 50 mg/L $FeSO_4$ $7H_2O$) (Chilton *et al.,* 1974) supplemented with 500 mg/L streptomycin and 50 mg/L Kanamycin and were incubated at 28°C for 16 hours shacking at 180 rpm. 3 mL of this culture were inoculated in 50 mL of fresh AB medium supplemented with the same antibiotics concentration and 2% glucose. This inoculum was incubated again at 28°C for 16 hours shacking at 180 rpm. Agrobacterium cells were collected through centrifugation at 4000 rpm for 10 min at RT ad suspended in 25 mL of AB medium. This step was repeated twice, the pellet was suspended in 10 mL of AB medium and a culture diluition 1:2 was used to measure $absorbance_{600nm}$. Cells were precipitated again and the pellet was finally suspended in a medium volume adjusted to get $absorbance_{600nm}$ =0.6.

*2.3.4 Co-cultivation and explants regeneration*

Tomato cotylendons were cut in ~5 mm explants. 32 of them were used as control explants and excluded of co-cultivation; they were immerged in AB medium for 10 min and divided into two groups: 16 of them were transferred on fresh GCF10 plates, the others 16 were positioned on the same plates used for co-cultivated explants. For the co-cultivation, explants were immerged in the Agrobacterium culture for 10 min, dried on sterile Whatman sheets and positioned on GCF10 supplemented with 375 µM acetosyringone for 2 days at 26°C in the dark. Explants were, then, transferred on GCF10 supplemented with 500 mg/L Carbenicillin and 40 mg/L Kanamycin and were incubated in a growth chamber at 26°C with a photoperioud of 16:8 h light/dark. Each week explants were transferred on fresh medium and at the beginning of calli formation, GCF11 supplemented with 500 mg/L Carbenicillin and 40 mg/L Kanamycin was used. Shoots produced in this way were let to take root on TRI2 supplemented with 250 mg/L Carbenicillin and 25 mg/L Kanamycin. Potential rooted transformants, identified by the ability to grow on kanamycin-containing medium, were acclimated in pots with sterile soil and transferred to a environmental chamber at 25°C with a

photoperiod of 16:8 h light/dark. After 3 weeks plants were usually transferred in 9 cm diameter vessels with sterile soil supplemented with substrate S (Floragard) and enriched with Nitrophoska®. After growing up and fruits maturation, tomatoes were collected for seeds isolation. Seeds were incubated over night in $3^0/_{00}$ HCl, then were washed with water, dried on Whatman sheets and finally stored at 4°C. To obtain next generations, seeds were let to germinate on a wet sterile Whatman sheet in a Petri plate in a vitro chamber at 25°C in the dark for about 1 week and then transferred in plateau with sterile soil as described before.

## 2.4 Molecular screening of transgenic plants

Transgenic plants were screened by PCR amplification using as template genomic DNA isolated from transgenic and control leaves as previously described.

### *2.4.1 RNA isolation and quantification*

Total RNA was prepared from leaves by a phenol/chloroform extraction and a lithium chloride precipitation. In order to extract a high quality RNA, fully expanded leaves of 3-4 weeks old-plants were cut and immediately frozen in liquid nitrogen. 0.5 g of leaves were powdered in nitrogen liquid using mortars and pestles. 750 µL of RNA extraction buffer (100 mM Tris-HCl pH 8.5, 100 mM NaCl, 20 mM EDTA pH 8.0 and 1% SDS) and 750 µL phenol/chloroform 1:1 were added to leaf powder, immediately vortexed and centrifuged at 13000 rpm at 4°C for 5 min. Phenol/chloroform extraction was repeated two times on the aqueous phase and then a chloroform extraction was carried out in the same conditions. Nucleic acid precipitation was obtained by adding 750 µL of isopropanol, incubation in ice for 5 min and centrifugation at 13000 rpm at 4°C for 10 min. Supernatant was removed and the pellet was, firstly, dried and then suspended in 400 µL of DEPC-treated water (DEPC- Diethylpyrocarbonate; Sigma). RNA selective precipitation was obtained through the addition of 1 volume of 4M Lithium Chloride (Sigma) and incubation on ice over-night. Samples were centrifuged at 13000 rpm at RT for 20 min, supernatant was discarded and pellet was suspended in 400 of DEPC-treated water. The addition of 0.1 volume of 3M Sodium Acetate pH 7.2 and 1 volume of

96% ethanol, the incubation at -80°C for 10 min and the centrifugation at 13000 rpm at 4°C for 10 min promote the precipitation of RNA. Pellets were finally suspended in 42 µL of DEPC-treated water. A 1:100 dilution of isolated RNA was quantified by measuring absorbance using Biophotometer (Eppendorf). RNA concentration was calculated using he following formula (1):

$$[1]\ 1\ OD_{260nm} = 40\ \mu g/mL$$

RNA integrity was checked by electrophoresis on a 1.2% agarose gel prepared without addition of Ethidium bromide. 5 µg of each sample were prepared with 20 µL of 10 X RNA Loading Buffer (400 µL Formamide, 120 µL 37% formaldeyde, 5 µL loading buffer 10X (par. 2.1.1), 1.2 µL 10 mg/µL ethidium bromide) and treated at 65 °C for 5 min. After denaturation, samples were loaded on the gel and a 50V potential difference was applied for 20 min. Gel visualization was performed as described in par. 2.1.1. Isolated RNA was treated with DNAse I to remove DNA contaminations. Two µg of RNA were added with 1X DNAse I Reaction Buffer (Invitrogen), 1 U DNAse I Amplification Grade (Invitrogen) and sterile water until a final volume of 10 µl. After the incubation at RT for 15 min, reaction was stopped by adding 1 µl of 25mM EDTA and heat treatment at 65°C for 10 min.

### *2.4.2 RNA retrotranscription and RT-PCR*

First strand-cDNA synthesis was performed using SuperScript II Reverse Transcriptase™ (Invitrogen) following this procedure: addition of 250 mM oligo dT primer, 0.5 mM dNTP mix and heating at 65°C for 5 min; quick chilling on ice and collection of tubes content by brief centrifugation; addition of 1X First Strand Buffer, 10 mM DTT, 40U RNAse OUT™ and incubation at 42°C for 2 min; after the addition of 200 U SuperScript II RT™ mix was still at 42°C for 60 min and reaction was finally stopped at 70°C for 15 min. The amplification of the cDNA region coding for EF-1α gene, a ubiquitously expressed gene (Shewmaker *et al.,* 1990), was performed as control of cDNA synthesis and of DNA contamination presence since primers used for the PCR reaction, StEF Fw and LeEF Rv (Table 1), are localized in two contiguous exons (Corrado *et al.* 2007). PCR products were visualized by electrophoresis as described in par. 2.1.1. RT-PCR was carried out

on 1 µl of cDNA using primer BBSBB Fw and RbcS Rv (Table 1) applying the same experimental conditions described in 2.1.2.

*2.4.3 Western blot analysis*

Total soluble proteins were isolated from leaves by powdering 0.5 g of tissue in liquid nitrogen using mortars and pestles; leaf powder was suspended in 300 µL of extraction buffer (6 M Urea, 50 mM Tris-HCl pH 7.5, 50 mM NaCl, 5 mM EDTA pH 8) and vortexed for 1 min on ice. After centrifugation at 13000 rpm at 4°C for 20 min, upper phase containing proteins was collected and transferred on a new eppendorf tube. Protein concentration was determined by the Bradford method (Bradford MM., 1976) using bovine albumin protein as the standard. 50 µg of total isolated proteins were separated by SDS-PAGE on a Mini-Protean II mini-gel apparatus (Bio-Rad), using 6% w/v stacking polyacrylamide gel and 12% w/v separation gel (Laemmli, 1970). Gel was stained with Comassie (0.1 % w/v R250 Brillant Blue, 40% Methanol, 10% Acetic Acid) over-night, then shaked in destaining solution (30% Acetic Acid, 40% Ethanol) for 3 hours, fixed in 10% acetic acid over-night and finally washed 3 times in distilled water for 1 h. Gel was dried by oven-like air drying system with a vacuum pump. After assessing proteins quality and integrity, 60 µg of extracted proteins were loaded on a new gel. Separated proteins were transferred onto nitrocellulose membrane by electroblotting with Mini Trans-Blot Cell (Bio-Rad). The blot was probed with the anti-ProSys polyclonal antibody as a primary antibody (dilution 1:1000) and anti-rabbit IgG conjugated with peroxidase (Santa Cruz Biotechnology) as a secondary antibody (dilution 1:2500). Prosystemin was visualized through a chemiluminescent detection system (ECL, GE&Healthcare) using Hyperfilm™ ECL (GE&Healthcare) and its molecular weight was estimated through comparison with PageRuler™ Plus Prestained Protein Ladder (Fermentas).

## 2.5 Bioassays

### *2.5.1 Spodoptera littoralis bioassay*

*S. littoralis* larvae were grown in an environmental chamber at 25°C with RH 70% under 16:8 light/dark photoperioud on artificial diet composed by 41.4 g/L wheat germ, 59.2 g/L brewer's yeast, 165 g/L corn meal, 5.9 g/L ascorbic acid, 1.8 g/L methyl 4-hydroxybenzoate, 29.6 g/L agar. Fourth instar larvae were transferred into plastic boxes containing vermiculite for pupae development. Leaf discs of transgenic (RSYS24, RSYS32, RSYS17) and control plants were daily supplied to experimental groups of 40 newly hatched larvae of *Spodoptera litoralis* and maintained at 28°C in plastic trays, containing a thin layer of a 2% agar solution, and closed with transparent plastic covers (CD International). Larvae were weighted everyday starting on day 3 from the beginning of the bioassay and mortality was daily checked during the whole larval feeding period. Statistical analyses were performed with the Graphpad Instat 3.0 software.

### *2.5.2 Aphid longevity and weight increase assay*

*Macrosiphum euphorbiae* colonies were continuously reared on *Solanum lycopersicum* cv. "San Marzano" in an environmental chamber at 20±1°C, 65±5% RH and a photoperiod of 16:8 hr light/dark. Four weeks after sowing, 40 RSYS24 and 40 Red Setter untransformed plants were infested with a newly born first instar nymph of *M. euphorbiae*. Assays were carried out at 20 ± 1°C, 65 ± 5% RH, 16:8 hr light/dark photoperiod. For the longevity assay, the presence of aphid or exuviae were monitored daily for a period of 20 days. The average age of aphids on RSYS24 plants were compared to those on Red Setter plants by t-Student's test. For the weight increase assay, 20 adult aphids were fed for 48 h on 3 biological replicates of Red Setter, RSYS24 and RSYS17 genotypes. Aphids were collected in a eppendorf tube and their weight was measured before and after feeding to calculate the weight increase. Mortality and reproduction rate were also calculated. Statistical analyses were carried out with one-way ANOVA (Duncan test) using SPSS software.

*2.5.3 Botrytis cinerea bioassay*

*B. cinerea* spores were cultivated on MEP solid medium (30 g/L malt extract, Oxoid; 5 g/L mycological peptone, Oxoid; 8 g/L bacto agar, Applichem) spreading on the media plates 20 µL of conidial suspension with the concentration of $1 \cdot 10^6$ conidia/mL and incubating at 22°C under diffused light for 15 days. Spores were suspended in sterile distilled water, filtered through sterile Kimwipes (Kimberly-Clark) to remove hyphal fragments and adjusted to a concentration of $1 \cdot 10^6/10^7$ conidia/mL. An aliquot of 10 µL of the spore suspension was applied between the leaf veins, at four different inoculation points per leaf. For this bioassay, four RSYS24 and RSYS17 plants were inoculated with fungi spores and incubated in a growth chamber at 23°C, under 16:8 h light/dark photoperiod and 90% RH. The size of the lesions was measured after 48 and 96 h. Lesions dimensions were measured using a digital caliber; diameters measured were used to calculated necrosis areas as elliptic areas and data significance was evaluated by one-way ANOVA analysis (Instat3 software).

## 2.6 Gene expression analysis

*2.6.1 Real Time RT-PCR*

Real Time RT-PCR was performed using Corbett Rotor Gene 6000 (Corbett Research). Reactions (total volume 10 µL) were prepared with 5 µL of the SYBR Green PCR Kit 2X (Qiagen), 0.3 µM of each primer, 1 µL of 1:20 diluition of first strand cDNA template. Amplifications were carried out using 2 technical and 3 biological replicates. The thermal cycling program started with a step of 10 min at 95°C, followed by 45 cycles of a 30 sec step at 95°C, 30 sec at $T_a$ temperature (calculated as $T_a = T_m - 5$, but often using a $T_a$ gradient PCR), 15 sec at 72°C, followed by a dissociation kinetic analysis to assess the specificity of amplification reaction. Primers, designed with the aid of the Primer Express 2.0 software (Applied Biosystem, Foster City, CA) were chosen to amplify a fragment of approximately 100 bp. Relative quantification of gene expression was carried out using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), where $\Delta Ct = Ct_{target\ gene} -$

$Ct_{reference\ gene}$. The housekeeping gene EF-1α was used as an endogenous reference gene for the normalization of the expression levels of the target genes. The amplification of EF-1α interested the region delimited by EF Fw Rt and EF Rv Rt primers (Table 2). The statistical significance of the results was evaluated using the t-Student's test. Genes under investigation include *prosystemin* (acc. num. M84800), *leaf wound-induced proteinase inhibitor I* and *II* (*InhI* and *InhII*; acc. num. K03290 and K03291), *metallo-carboxypeptidase inhibitor* (*MCPI*; acc. num. X59282), *arginase* (acc. num. AY656837), *mate efflux* (acc. num. BI933305), *LoxD* (acc. num. U37840), *subtilisin* (acc. num. TA38526_4081), *SAM* (acc. num. ES894405), *threonine deaminase* (acc. num. M61914), *osmotin* (acc. num. AY093595), *Pto locus* (acc. num. TC223474), *pathogenesis-related protein 10* (acc. num. AK329477), *kunitz protease inhibitor* (acc. num. X73986), *JA-ZIM* (acc. num. AK327683), *polyphenol oxidase* (acc. num. BI925947).

*2.6.2 Microarray analysis*

Samples were obtained from two RSYS lines (RSYS24 and RSYS32), chosen based on their transgene expression levels, and Red Setter untransformed plants, each one in three biological replicates. Leaf tissues from 4 weeks-old seedlings were powdered in liquid nitrogen as described above and homogenised in Qiazol solution (Qiagen). RNA was extracted using Plant RNeasy mini kit (Qiagen) according to manufacturer's protocol. RNA samples were analyzed quantitatively and qualitatively by NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies) and by Bioanalyzer (Agilent Technologies). Total RNA from transgenic and control samples was amplified in the presence of cyanine-3/cyanine-5 labeled CTP using Agilent low RNA Input Agilent's Quick Amp Labeling kit for two-color (Agilent Technologies) according to manufacturer's protocol. All samples were processed together with Agilent's RNA spike kit. After labeling, samples were purified using RNeasy mini spin column (Qiagen) to remove unincorporated dye-labelled nucleotides. The quality of labeled targets was determined by calculating the amount of cDNA produced, the pmoles of dye incorporated and the frequency of incorporation by NanoDrop. Equal amounts of cRNAs (825 ng) from control (labeled with Cy3) and from transgenic samples (labelled with Cy5) were mixed together and hybridized to the Agilent's 4x44k

tomato chips in a hybridization, on which 43803 probes corresponding to tomato ESTs are spotted. Chip was incubated in a oven at 65°C for 17 hours with rotation at 10 rpm using solution provided in Agilent's Gene Expression Hybridization kit according to manufacturer's recommendations. After hybridization slides were washed with Gene Expression Wash buffer 1 for 1 minute at room temperature and Gene Expression Wash buffer 2 for 1 minute at 37°C. Finally to dry the slides and prevent ozone degradation arrays were treated with the Stabilization and Drying Solution (Agilent Technologies) for 30 seconds at room temperature. After washing, slides were scanned with the Agilent's dual laser microarray scanner (G2565AA) and image data were processed using Agilent Feature extraction software (FE) (Agilent Technologies). This software calculates log ratios and p-values for valid features on each array and provides a confidence measure of gene differential expression performing outlier removal and background subtraction. Furthermore, FE filters features that are not positive and significant respect to background and/or saturated. FE was also used to perform linear and LOWESS dye normalization to correct dye bias. This software also performed the spike probes hybridization efficiency analysis comparing the expected The raw data and associated sample information were loaded and processed by GeneSpring GX 10 (Agilent Technologies). Statistical analysis was performed using background- corrected mean signal intensities from each dye channel. Microarray data were normalized using intensity-dependent global normalization (LOWESS). Differentially expressed RNAs were identified using a filtering by the Benjamini and Hochberg False Discovery Rate (p-Value<0.05) to minimize selection of false positives. Of the significantly differentially expressed RNA, only those with greater than 2-fold increase or 2-fold decrease in expression compared to the controls were used for further analysis. Microarray results were verified by Real Time RT-PCR targeting 14 defence genes. Primers used are listed in Table 1. The correlation analysis between microarray and Real Time data was carried out by the conversion of microarray fold change and RQ in $\log_2$ values and visualizing them on a dispersion xy graph. Tendency line and $R^2$ value were used to evaluate the correlation.

*2.6.3 Functional annotation*

The Agilent's 4x44k tomato array (43803 probes) is based on known tomato genes, but also on annotated ESTs and cDNA sequence information. Differentially expressed sequences found in this study were re-annotated using different methods to get a proper functional annotation for the unknown gene names. Fasta format sequences were downloaded from NCBI, The Tomato Gene Index (DFCI) and Plant Transcript Assemblies Database (TIGR). Functional annotation was performed using Blast2GO software (CIPF, Valencia; www.blast2go.org) enriching the analysis by loading Kegg pathways. Pathways analysis on differentially expressed sequences was also carried out using Paintomics software (CIPF, Valencia; www.paintomics.org) submitting the corresponding Arabidopsis protein identifiers. These identifiers were obtained by blastx querying the Arabidopsis RefSeq_protein database applying a filter (e-value Exp Max=$10^{-5}$) and collecting only the first hit for each query. RefSeq protein codes were translated in TAIR (The Arabidopsis Information Resource) accession number using the Identifiers Converter available in Babelomics 4.2 (www.babelomics.org) that were used for the pathways analysis.

*2.6.4 PPI network*

RSYS differentially expressed sequences were subjected to PPIs (protein-protein interaction) analysis using their corresponding Arabidopsis identifiers by The Arabidopsis Information Resource (TAIR) (www.arabidopsis.org). Networks were built using the Arabidopsis Interactions Viewer available at University of Toronto website (http://bar.utoronto.ca/). The network.sif file was downloaded from this tool and was used to filter the Arabidopsis annotation file, containing all Arabidopsis protein annotations and downloaded from TAIR website (www.arabidopsis.org), in order to build an attribute file which consists in the description of each node in the network. The network obtained was enriched in the number of nodes (proteins) coming from RSYS list using as reference an interactome downloaded from TAIR website (www.arabidopsis.org). For this job the editor Emacs with R programming language and igraph library which contains the shortest path function were used. So further nodes were attached on the previous network matching the RSYS list with the new interactome from TAIR using the shorest path function allowing two

intermediate nodes. All nodes included in the network were described by an attribute file obtained from all Arabidopsis annotation available at the TAIR website. The network and the attribute file were imported in Cytoscape software (www.cytoscape.org) in order to paint, analyze and integrate interactions. The parameters that describe the network were studied using the graph theory. The main parameters analyzed were:

- **Connections degree** refers to the number of edges (interactions) that are connected to a node (protein).
- **Betweenness** indicates the centrality of a node in the network and it is described by the number of shortest paths passing through a node, where "shortest path" stands for the minimum path (sequence of edges) connecting two nodes.
- **Clustering coefficient** evaluates how much a node is connected to its neighborhood (all neighbors of a node *n*) and it is described by the ratio between the number of edges between *n* and its neighbors on the maximum number of edges that can possibly exist between and its neighbors.

The parameters analysis were performed using the Network Analyzer tool in Cytoscape software. The statistical significance of these parameters was evaluated with the SNOW software available at Babelomics website providing the interactome from TAIR as reference and submitting the Arabidopsis TAIR ID list.

## 2.7 Expression analysis of defense genes after biotic stimuli

### *2.7.1 Spodoptera littoralis induction*

*S. littoralis* larvae were grown as described in par. 2.5.1. For this experiment, a single four instar larva was let to feed on tomato leaf for one hour and then removed. Leaf samples were collected at several time points: 15 min, 30 min, 1.5 h, 3 h, 6 h, 9 h and 24 h starting from the moment in which *Spodoptera* larva was positioned on the leaf. After one feeding hour it was removed continuing to collect samples at the established time points. Collected samples were immediately frozen in liquid nitrogen and used for RNA isolation and gene expression analysis. 3 biological replicates for each experimental point of Red Setter, RSYS24 and

RSYS17 plants were used. The analysis involved *prosystemin* gene (acc. num. M84801) and *leaf wound-induced proteinase inhibitor I* (acc. num. K03290) and was carried out on local and distal leaves as described in par. 2.6.1. For each genotype, time 0 samples were used as calibrator.

### *2.7.2 Aphid infestation*

A time course infestation of 'Red Setter' plants with *M. euphorbiae* was performed to study the expression pattern of defence genes. Adult aphids (15) were left on 'Red Setter' leaves and the for 24, 48 and 96 h. Each experimental point had 3 biological replicates. RNA was isolated from each sample and cDNA was synthesized as described in par. 2.4.2. Real Time RT-PCR and data statistical analysis were performed as described in par. 2.5.1 using sample at time 0 (without infestation) as calibrator. Genes under investigation are: *prosystemin* (acc. num. M84800), *leaf wound-induced proteinase inhibitor I* and *II* (*InhI* and *InhII*; acc. num. K03290 and K03291), *treonine deaminase* (acc. num. M61914), *pathogenesis-related protein 1* (acc. num. X71592), *kunitz protease inhibitor* (acc. num. X73986), *LoxD* (acc. num. U37840), *leucine aminopeptidase* (acc. num. U50152), *wrky 40* (acc. num. AK325041) Primers used for their expression analysis are in table 2.

### *2.7.3 Botrytis cinerea infestation*

Defence genes induction by *B. cinerea* infection was carried out reproducing the same experimental conditions and procedures used for the bioassay described in par. 2.5.3. A time-course expression analysis was performed on Red Setter plants using 3 biological replicates for each experimental point and time 0 sample as calibrator for Real Time RT-PCR. Genes under investigation were: *prosystemin* (acc. num. M84801), *leaf wound-induced proteinase inhibitor I* (acc. num. K03290), *osmotin* (acc. num. AY093595), *Pti5* (acc. num. U89256), *lipoxygenase A* (SGN-U143303), *Miraculin* (SGN-U144553), *Extensin* (acc. num. X55688), *arginase 2* (acc. num. AK321112). Relative expression and statistical analysis were carried out as described in par. 2.5.1.

# 3. RESULTS

## 3.1 Genetic background evaluation

*Solanum lycopersicum* cv. “Red Setter” was chosen as suitable genetic background to evaluate prosystemin effect in tomato responses against aphids as the cultivar lacks the major aphid resistance gene. This genetic condition was also verified by CAPS analysis to assess the absence of dominant alleles at *locus Mi*, known to be involved in resistance against nematodes and the potato aphid *Macrosiphum euphorbiae* (Rossi *et al* 1998). *Mi* status was checked through the analysis of the marker REX-1, a DNA marker tightly linked to *Mi* gene (Williamson *et al*., 1994). After the amplification of a 750 bp fragment containing both susceptible and resistant Mi alleles, the amplicons are treated with *Taq*I restriction enzyme. Homozygous (R/R) genotypes originate two fragments (570 and 160 bp), while susceptible (r/r) genotypes, do not undergo any restriction. Amplicons obtained from heterozygous genotypes originate three fragments ( 750, 570 and 160 bp). Results obtained with DNA extracted from 'Red Setter' leaves are shown in fig. 4. DNA isolated from *S. lycopersicum* cv. “Motelle” leaves was used as positive control, since this cultivar is homozygous for the dominant allele at *Mi locus* (Cooper *et al*., 2005).

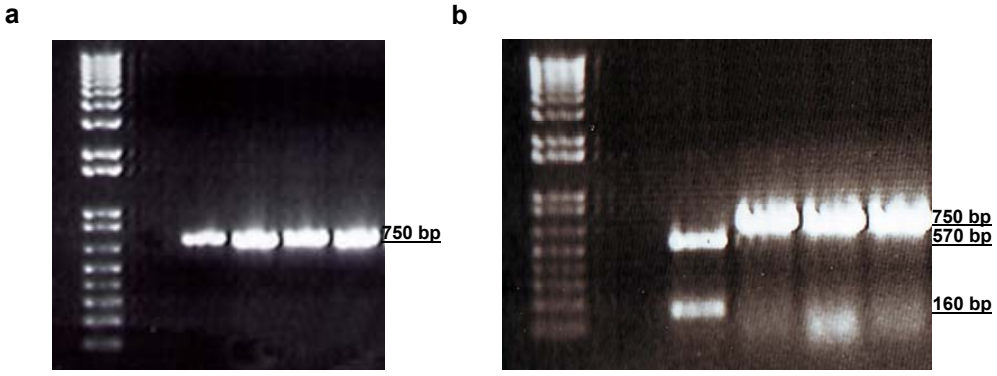


**Figure 4. The image displays the CAPS-REX1 analysis. a) Electrophoresis on a 1.5% agarose gel of PCR product with Mi REX1 primers; b) TaqI digestion products; lane 1: 1 Kb Plus (Invitrogen), lane 2: no template control, lane 3: cv. Motelle DNA, lane 4-6: cv. Red Setter DNA.**

This analysis confirmed that 'Red Setter' genome allocate only the susceptible allele at *Mi locus.* The tomato resistance gene *Mi-1* encodes a protein with CC-NBS-LRR motifs and it is the unique cloned R gene involved in the resistance

against nematodes and aphids (Milligan *et al.*, 1998). *Mi* was introgressed into cultivated tomato (*Lycopersicon esculentum*) from its wild relative *Lycopersicon peruvianum* (Kaloshian *et al.,* 2000). Many modern tomato cultivars resistant to nematodes carry this gene, such as “Motelle” and “Sun6082”. The choice of the susceptible “Red Setter” was due to the need of a suitable background in which evaluate only ProSys effect in tomato-aphid interactions.

## 3.2 Genetic trasformation of tomato plants for ProSys costitutive expression

### *3.2.1 Plasmid control*

The pMZ plasmid used for tomato genetic transformation was checked for the presence of transgenic cassette. This binary vector (Fig. 1) carries the RSYS cassette in which there is the full length *prosystemin* cDNA (ProSys) under the control of the constitutive promoter CaMV $35S^2$ and RbcS terminator. The transgenic cassette and the PCR strategy used for the screening is shown in fig. 5.

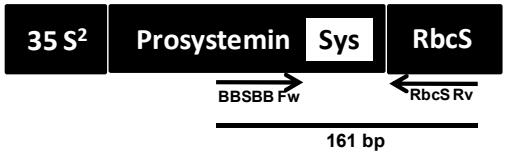


**Figure 5. RSYS cassette is contained in pMZ plasmid and used for tomato genetic transformation to produce the homonymous plants. The image also shows the PCR strategy used.**

In order to check the transgenic cassette and to assess its presence in the *Agrobacterium* frozen to use for tomato transformation, plasmid DNA was isolated from (At)pMZ frozen cells (Rocco *et al.,* 2008). The plasmid quantification was performed by comparison with known quantities of λ fagus DNA (Fig. 6a). The presence of RSYS cassette and the integrity of the transgenic construct were checked by PCR. The elechtrophoresis revealed PCR products of the expected size (Fig. 6b).

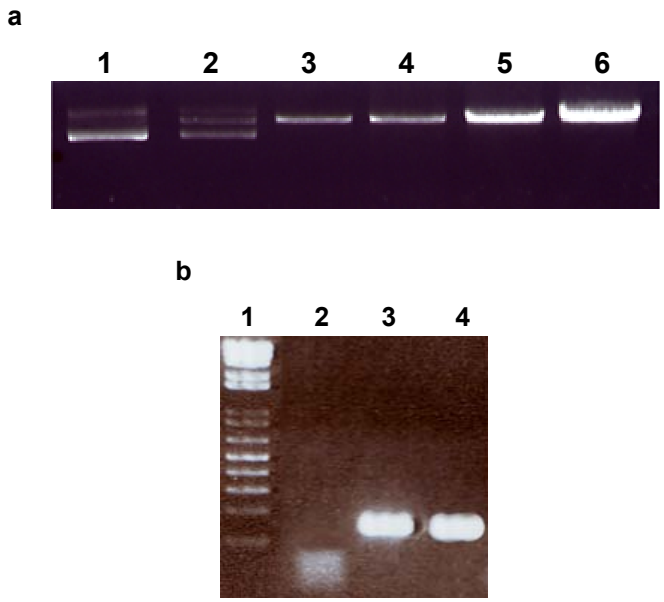


**Figure 6. a) Quantification of the isolated plasmid by comparison with fagus λ DNA; lane 1: pMZ-A, lane 2: pMZ-B, lanes 3-6: fagus λ DNA (25, 50, 100, 200 ng); b) Electrophoresis of PCR products; lane 1: 1 Kb Plus Ladder (Invitrogen), lane 2: no template control, lane 3-4: agarose gel elechtrophoresis of amplicons on pMZ plasmids.**

(At)pMZ stored cells were confirmed to contain the binary vector and the expression cassette with the transgene, so ready to use for tomato genetic transformation.

### *3.2.2 Transgenic plants production*

*Solanum lycopersicum* cv. "Red Setter" was permanently transformed via *Agrobacterium tumefaciens* with pMZ plasmid to produce tomato plants constitutively expressing the full length of the prosystemin cDNA. About 800 explants were used for the genetic transformation and subjected to the co-cultivation with agrobacterium cells. Control explants were not treated with agrobacterium: for the selection control, explants were positioned on the transformation media supplemented with selective antibiotics; for the regeneration control, explants were positioned on the transformation media without antibiotics. Transformation controls are important to assess the antibiotics selection and the media regeneration effectiveness. Co-cultivated explants produced calli and sprouts that showed no phenotypical differences compared to controls. Figure 7 shows an example of explants and controls in an intermediate step of the genetic transformation.

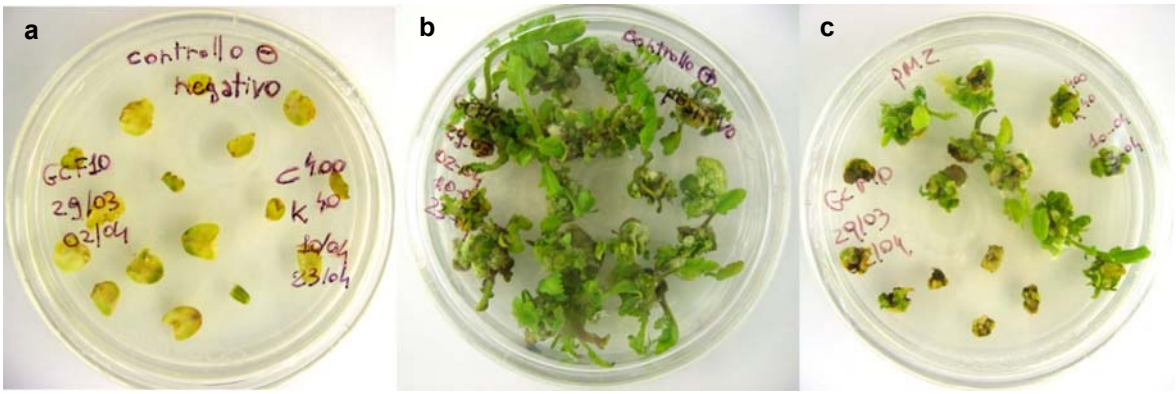


**Figure 7. The image displays explants and calli in an intermediate phase of agrobacterium-mediated transformation. a) selection control; b) regeneration control; c) example of explants on 50 mg/L Kanamycin medium.**

From each explant a single callus was obtained and from each callus only one sprout was cut and positioned on the rooting media selecting, in this way, only single independent transformation events. Putative transgenic plants were transferred in sterile soil and named RSYS using progressive numbers to identify each putative transformant.

## 3.3 Molecular screening of transgenic plants

### *3.3.1 PCR screening*

Putative transformants were screened by PCR to verify the presence of the transgenic cassette. The strategy used for these screenings is the same used to check pMZ plasmid. Figure 8 shows an example of the quantification of genomic DNA isolated from putative transgenic and control leaves compared with known quantities of λ fagus DNA (Promega).

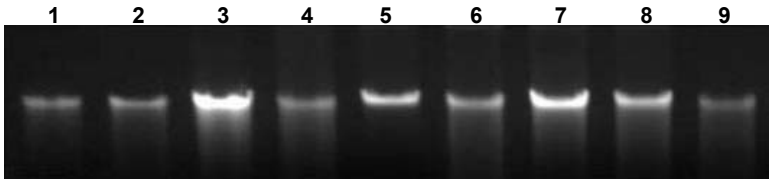


**Figure 8. Quantification of genomic DNA isolated from tomato leaves by comparison with fagus λ DNA. Lane 1-3: 50, 100, 200 ng of λ DNA (Promega); lane 4-6: genomic DNA from Red Setter leaves; lane 7-9: genomic DNA from RSYS leaves.**

Isolated genomic DNA was used as template for PCR screening using primer BBSBB Fw and RbcS Rv (Table 2). Gel elechtrophoresis of the 161 bp PCR products obtained for transgenic plants, from now on referred as RSYS samples, are shown in fig. 9.

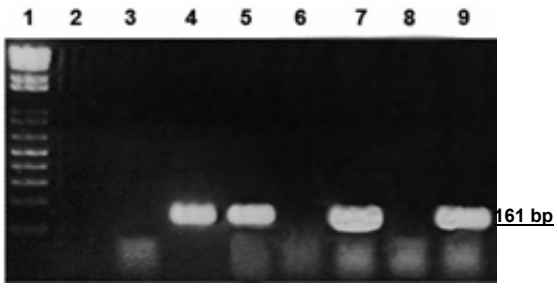


**Figure 9. The image displays the elechtrophoresis on 2% w/v agarose gel of PCR products. Lane 1: 1 Kb Plus Ladder (Invitrogen); lane 2: no template control; lane 3: PCR product on Red Setter DNA; lane 4: PCR products on pMZ plasmid used as positive control; lane 5-9: amplicons on RSYS genomic DNA.**

PCR screening on transgenic plants $T_0$ generations underlined 17 positives for RSYS. Transformation efficiency was calculated by dividing the number of positive plants on the number of starting explants and was 2.36% for this transformation.

### *3.3.2 Transgene expression analysis*

In order to verify transgene expression, analysis at RNA and protein levels were carried out on transgenic and control samples. Total RNA was isolated from fully expanded Red Setter and RSYS leaves and quantified by measuring absorbance using a spectrophotometer. An example of absorbance values obtained during RNA isolations are in table 4.

| Sample | $A_{230}$ | $A_{260}$ | $A_{280}$ | $A_{320}$ | $A_{260}/A_{230}$ | $A_{260}/A_{280}$ | [µg/µL] |
|---|---|---|---|---|---|---|---|
| **RedS 1** | 0.245 | 0.539 | 0.269 | 0.016 | 2.37 | 2.14 | 2.23 |
| **RedS 2** | 0.225 | 0.512 | 0.246 | 0.014 | 2.36 | 2.14 | 2.05 |
| **RSYS 24.1** | 0.357 | 0.823 | 0.393 | 0.011 | 2.35 | 2.13 | 3.29 |
| **RSYS 24.2** | 0.231 | 0.55 | 0.264 | 0.01 | 2.45 | 2.12 | 2.2 |
| **RSYS 32.6** | 0.195 | 0.438 | 0.213 | 0.014 | 2.34 | 2.13 | 1.75 |
| **RSYS 32.9** | 0.171 | 0.483 | 0.228 | 0.004 | 2.82 | 2.13 | 1.93 |

**Table 4. RNA absorbance values on some Red Setter and RSYS samples.**

Ratios of $A_{260nm}/A_{230nm}$ and $A_{260nm}/A_{280nm}$ let to evaluate RNA purity detecting the presence of carbohydrate, phenols, aromatic compounds looking at the first ratio while for protein contaminations the second one; $A_{320nm}$ instead is an index of turbidity. Optimal value for the $A_{260nm}/A_{230nm}$ ratio is around 2.2, while for the $A_{260nm}/A_{280nm}$ is around 2. RNA quality was checked by looking at the integrity and the intensity of rRNA bands after elechtrophoresis (fig. 10).

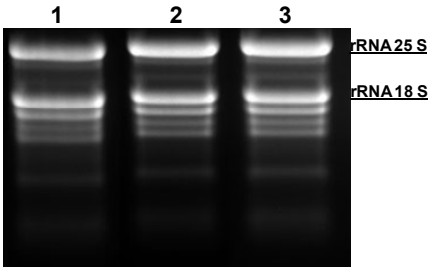


**Figure 10. Elechtrophoresis on a 1.2% w/v agarose gel of 5 µg of isolated total RNA from Red Setter (lane 1), RSYS 24 (lane 2) and RSYS 32 (lane 3) leaves.**

Isolated RNA was used for the first-strand cDNA synthesis after DNAse I treatment. cDNA was verified by PCR on EF-1α gene, expressed constitutively in tomato (Pokalsky *et al.,* 1989). Primer used for this analysis, StbEF Fw and LeEF Rv, are suitable not only to assess cDNA synthesis but also to detect any DNA traces, since they anneal on two contiguous exons determining amplicons of different lengths (Fig. 11).

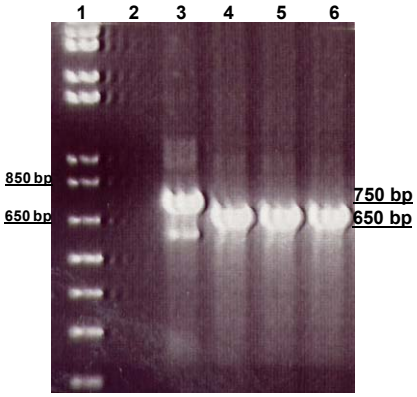


**Figure 11. Elechtrophoresis on a 1.5% w/v agarose gel of StbEF Fw and LeEF Rv PCR products on the synthesized cDNA. Lane 1: 1 Kb Plus Ladder (Invitrogen); lane 2: no template control; lane 3: PCR product on genomic DNA; lane 4: PCR product on Red Setter; lane 5-6: PCR products on two RSYS cDNA.**

No DNA contaminations were found in the synthesized cDNA samples so they were used as template for RT-PCR with BBSBB Fw and RbcS Rv primer to assess the presence of the transgene transcript (fig. 12).

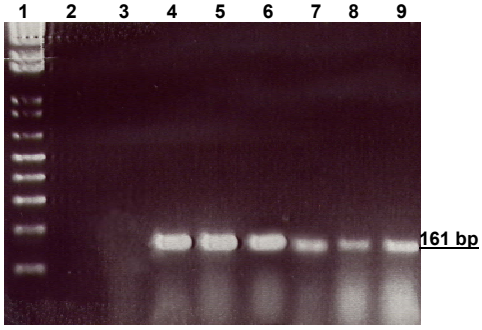


**Figure 12. Elechtrophoresis on a 2% w/v agarose gel of BBSBBFw and RbcS Rv PCR products on the synthesized cDNA. Lane 1: 1 Kb Plus Ladder (Invitrogen); lane 2: no template control; lane 3: PCR product on Red Setter cDNA; lane 4: PCR products on pMZ plasmid (+ control); lane 5-9: amplicons on some cDNA obtained from transgenic RNA.**

PCR products on RSYS cDNA showed the expected size (161 bp) comparable to the product obtained on pMZ plasmid used as positive control; all the lines expressed the transgene at transcriptomic level (fig. 12).

### *3.3.3 Relative quantification of transgene expression*

In order to estimate transgene expression levels in the population of transgenic plants , relative quantification of prosystemin transcripts were carried out by Real Time RT-PCR using 'Red Setter' untransformed plants as calibrator. For each cDNA two technical replicates were used comparing their average with 'Red Setter' one. Transgenic population showed a wide variety of *prosystemin* expression levels (fig. 13) allowing to choose some lines for further investigations according to their transgene expression levels.

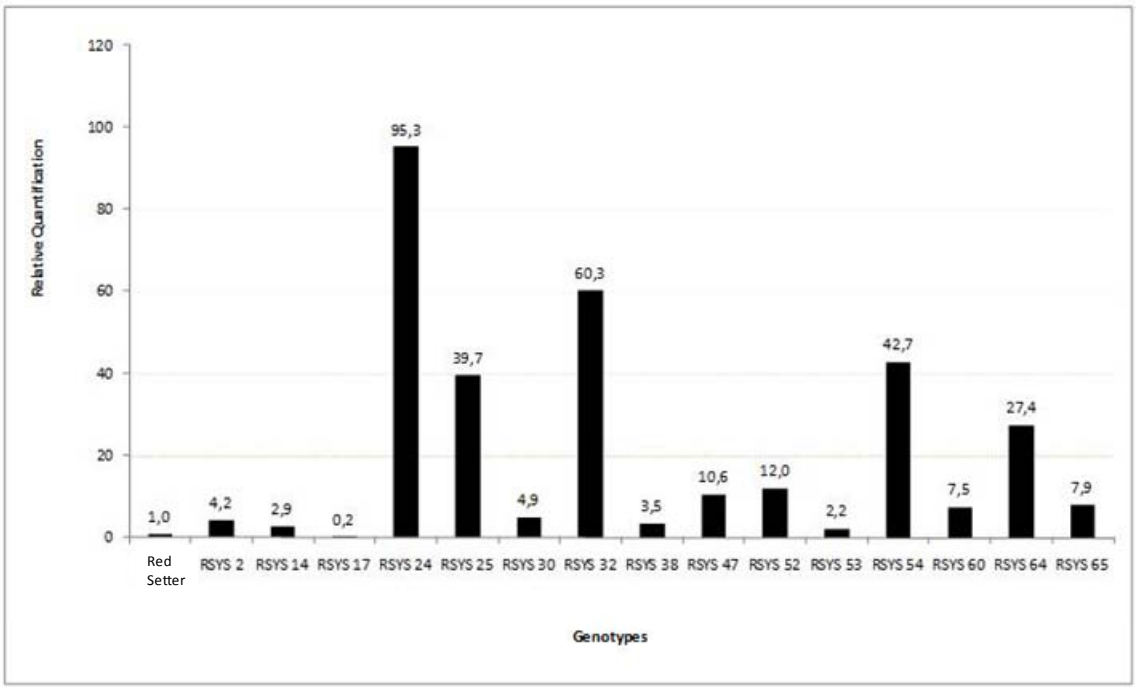


**Figure 13. Relative quantity of the target transcript in some RSYS $T_0$ plants. The observed ProSys level is the sum of endogenous and transgenic transcripts. Quantities (RQ) are shown relative to the calibrator 'Red Setter' genotype. On the y-axis is reported the RQ values on a linear scale.**

RSYS24 and RSYS32 were selected as they show two different expression level of the gene. RSYS17 was also selected as it represents a line in which the co-suppression of *prosystemin* occurred. Selected RSYS lines were let to self-pollinate, seeds were collected and used to get $T_1$ generations. $T_1$ transgenic plants were subjected to phenotypical analysis by comparing with 'Red Setter' plants. RSYS plants resulted reduced in their size showing a stunted appearance compared to the control (fig. 14).

**Figure 14. Transgenic and control plants. Starting from the left, 'Red Setter', RSYS24, RSYS32 and RSYS17 plants.**

*3.3.4 Molecular characterization of $T_1$ generation*

Selected RSYS lines were let to self pollinate to obtain $T_1$ generation that was screened by PCR as described in par. 3.3.1. Transgene expression analysis of $T_1$ generation was performed using 3 biological and 2 technical replicates for each genotype and results were consistent with expression analysis carried out on $T_0$ generation confirming transgene stability (fig. 15).

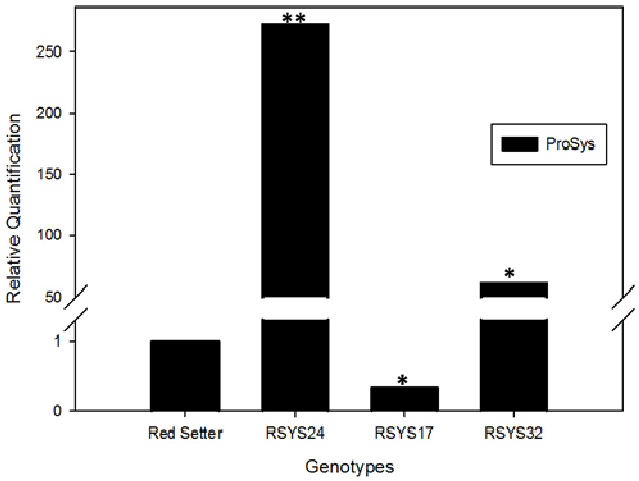


**Figure 15. Relative quantity of the target transcript in four transgenic genotypes (RSYS) and in control plant (RS). Quantities (RQ) are shown relative to the calibrator RS genotype. On the y-axis is reported the RQ values on a linear scale. Asterisks indicate that the $2^{-\Delta\Delta Ct}$ values were significantly different (*P<0.05; **P<0.01; Student's t-test).**

In order to check transgene expression at protein level, a western blot analysis was performed using a polyclonal anti-ProSys antibody, kindly provided by Dr. Andreas Shaller from University of Hohenheim (Fig. 16).

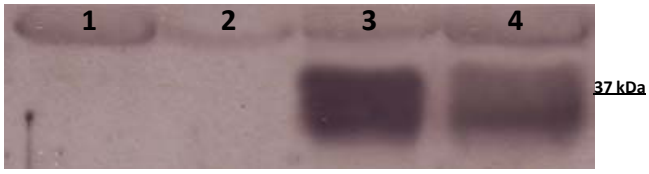


**Figure 16. Detection of Prosystemin protein in transgenic plants by Western blotting analysis. Lane1: total soluble protein extracted from control plant; lane 2-4: total soluble protein extracted from RSYS 17, 24, 32 plants, respectively.**

A protein band with an apparent molecular weight of 37 KDa was observed in RSYS 24 and RSYS32 samples, while no bands were visualized in RSYS 17 and 'Red Setter' samples, as expected. The observed molecular weight of prosystemin

is different from the real molecular weight that is 23 KDa. This incongruity was already underlined in previous experiments and explained as the consequence of the highly hydrophilic aminoacid content of the protein which affects its mobility (Delano *et al.,* 1999; Tortiglione *et al.,* 2003). Once assessed the transgene expression, its activity in tomato defence was also evaluated analyzing the expression of proteinase inhibitors genes that are known to be induced by ProSys (McGurl *et al.,* 1994). The verification of PIs induction by *ProSys* over-expression is necessary to assess that the systemic response in RSYS plants works correctly. This is an important assumption for the following investigations. This analysis was carried out by Real Time RT-PCR as described above in this paragraph, targeting 3 proteinase inhibitor genes: *leaf wound-induced proteinase inhibitor I* and *II* (*InhI* and *InhII*; acc. num. K03290 and K03291), *metallo-carboxypeptidase inhibitor* (*MCPI*; acc. num. X59282). Fig. 17 shows the relative expression analysis of these 3 genes in RSYS24, RSYS32 and RSYS17 lines compared to the calibrator, cultivar 'Red Setter'. As expected, these genes were strongly up-regulated in RSYS24 and RSYS32 while down-regulated in RSYS17, showing the same ProSys expression trend.

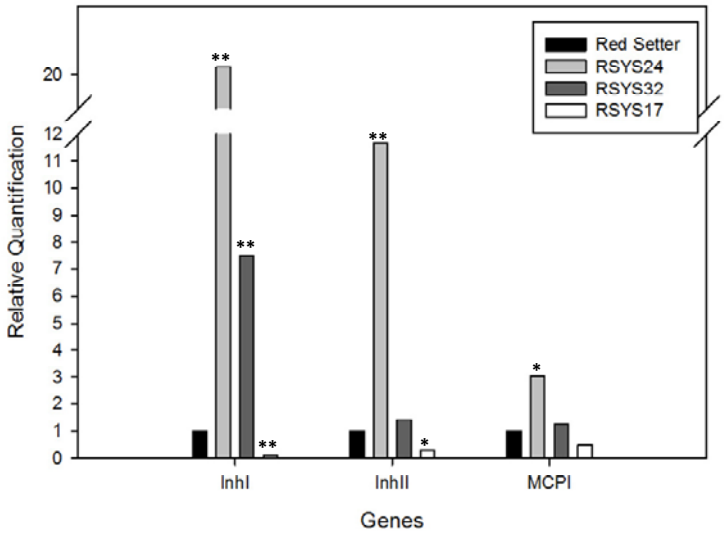


**Figure 17. Relative quantity of the PIs transcripts in four transgenic genotypes (RSYS) and in control plant (Red Setter). Quantities (RQ) are shown relative to the calibrator Red Setter genotype. On the y-axis is reported the RQ values on a linear scale. Asterisks indicate that the $2^{-\Delta\Delta Ct}$ values were significantly different (**P<0.01; *P<0.05; Student's t-test).**

It appears that the level of expression of *prosystemin* correlates with the level of expression of proteinase inhibitors.

### 3.4 Bioassays

In order to evaluate resistance or susceptibility conferred by ProSys over-expression against several biotic agents, RSYS plants were analyzed by bioassays against the lepidopteran *Spodoptera littoralis,* the aphid *Macrosiphum euphorbiae* and the fungus *Botrytis cinerea.* All insect assays were carried out in collaboration with professor Francesco Pennacchio workgroup at the Department of Agrarian Entomology and Zoology "Filippo Silvestri", while fungi assays were performed in collaboration with Dr. Michelina Ruocco at the Department of Arboriculture, Botany and Plant Pathology of University of Naples.

#### *3.4.1 Spodoptera littoralis assay*

The selected RSYS genotypes showed also different PIs expression levels (par. 3.3.3) that affect larvae feeding due to their strong anti-nutritional activity (McGurl *et al.,* 1994). The aim of this experiment was to verify the complete functionality of the ProSys-mediated signalling pathway in RSYS plants and to assay the effect of lines expressing different levels of PIs on larvae weight. Figure 18a shows *S. littoralis* larvae after 10 days of feeding on transgenic and control leaves, while the graph in fig. 18 b reports the weights of larvae at different days of trial.

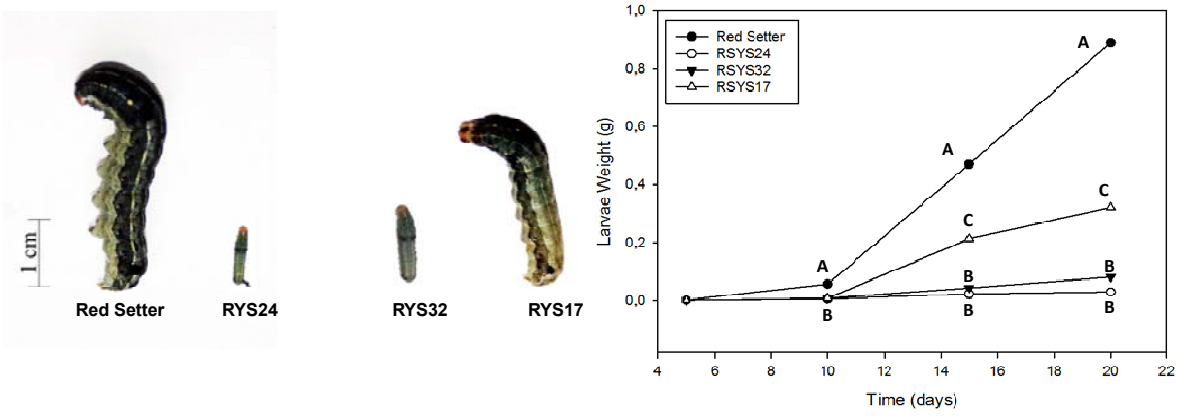


**Figure 18. Images display results of *S. littoralis* weight assay on RSYS plants. (a) *S. litoralis* larvae at 10 days of feeding on transgenic and control leaves. (b) Weight of larvae fed on leaf discs of transgenic and control plants. Letters indicate statistical groups (One-way ANOVA; Tukey test).**

Larvae fed on the two transgenic plants with high expression level of *ProSys,* RSYS24 and RSYS32, had a much reduced weight increase of larvae fed on control plants. The weights of larvae fed on RSYS24 and RSYS32 leaf discs were very much alike as confirmed by statistical analysis.

| | feeding days | | | |
|---|---|---|---|---|
| **Genotypes** | **5** | **10** | **15** | **20** |
| **Red Setter** | 1,5054 | 56,794 | 470,981 | 890,305 |
| **RSYS24** | 1,5076 | 6,01714 | 21,4983 | 28,887 |
| **RSYS32** | 1,5082 | 7,66238 | 42,8531 | 80,6583 |
| **RSYS17** | 1,5068 | 7,71368 | 213,278 | 321,318 |

**Table 5. Weights of *S. littoralis* larvae fed on transgenic and control leaves at 5, 10, 15, 20 feeding days.**

Larvae longevity was also registered and its trend was consistent with the weight one (fig. 19) since the observation of the maximum mortality, so the lowest longevity, on RSYS24 and RSYS32 (table 6).

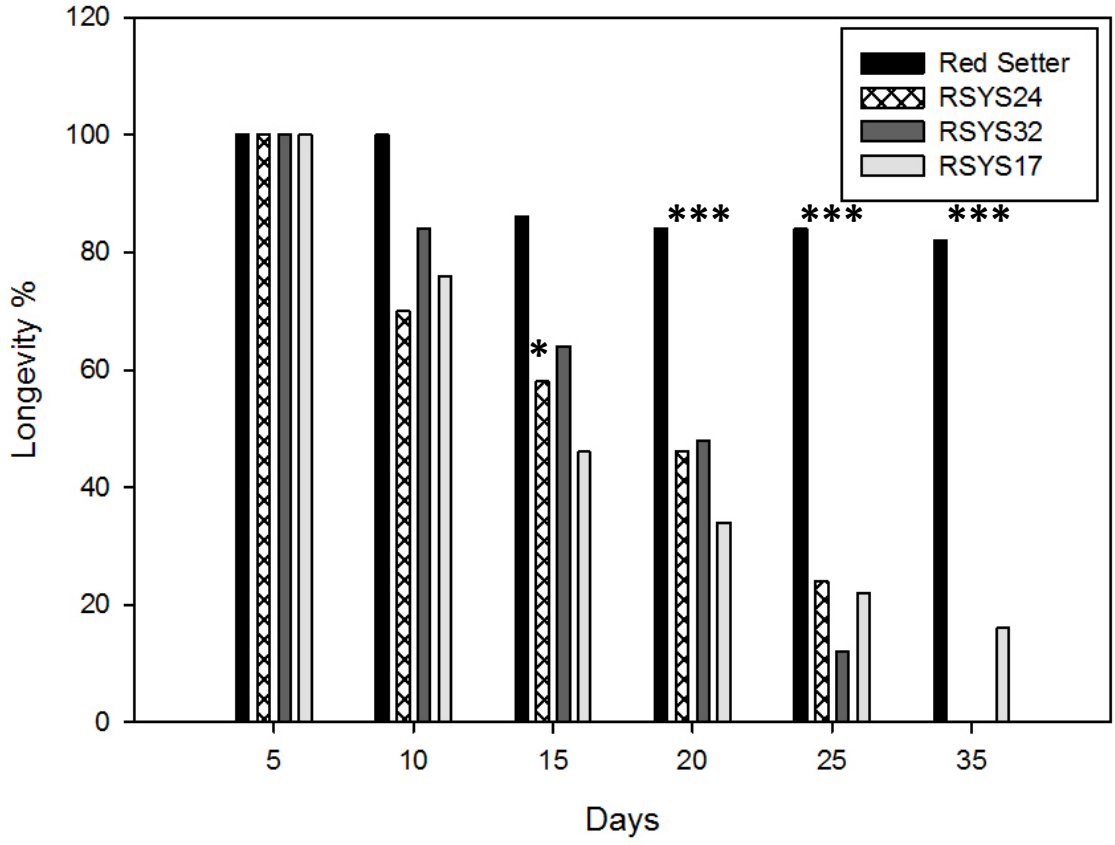


**Figure 19. Longevity of *S. littoralis* larvae fed on transgenic and control leaves. ***p<0.001; *p<0.05 (Chi-square test).**

Larvae fed on the co-suppressed line RSYS17 showed an intermediate weight between the other transgenic lines and controls, not consistent with expected results on a silenced line (fig. 18a). The visual observation was consistent with weight measurements (table 4), since their trend is intermediate between the control and the other 2 transgenic lines (fig. 18b). Larvae longevity on RSYS17 was similar to RSYS24 and RSYS32 lines being reduced compared to the control with the exception of the last days assayed (fig. 19; table 6).

| | feeding days | | | | | |
|---|---|---|---|---|---|---|
| **Genotypes** | **5** | **10** | **15** | **20** | **25** | **35** |
| **Red Setter** | 100 | 100 | 86 | 84 | 84 | 82 |
| **RSYS24** | 100 | 70 | 58 | 46 | 24 | 0 |
| **RSYS32** | 100 | 84 | 64 | 48 | 12 | 0 |
| **RSYS17** | 100 | 76 | 46 | 34 | 22 | 16 |

**Table 6. *S. littoralis* longevity percentages on transgenic and control leaves.**

### *3.4.2 Aphid bioassays*

ProSys involvement in responses against aphids was evaluated through 2 kinds of bioassay: longevity and weight assays. *Macrosiphum euphorbiae* was continuously reared on *Solanum lycopersicum* cv. "San Marzano" and was used to infest RSYS24, the transgenic genotype with the highest expression of *ProSys* gene. For the longevity assay, the presence of aphid or exuviae were monitored daily for 20 days. The average of aphids on RSYS24 plants were statistically different from those on Red Setter plants (fig. 20).

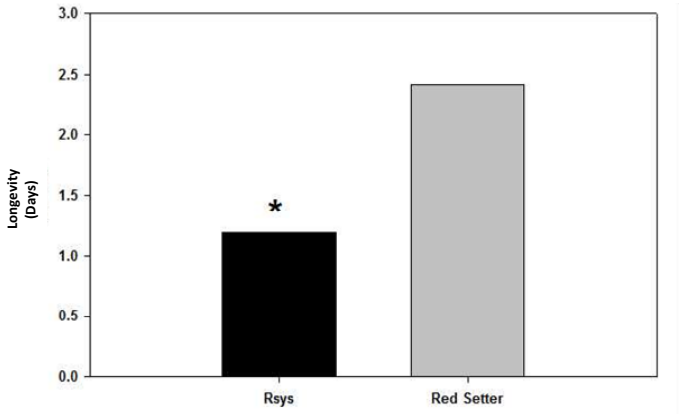


**Figure 20. Aphid longevity measured on RSYS plants (line 24) in comparison to the control. *p<0,05 (t-Student's test).**

A reduced aphid longevity was observed on RSYS24 plants compared to the control (fig. 20) suggesting that the ProSys signaling pathway could also affect phloem feeding insects. Similarly the weight of aphids reared on transgenic plants was strongly reduced respect that observed for aphids grown on control plants (fig. 21).

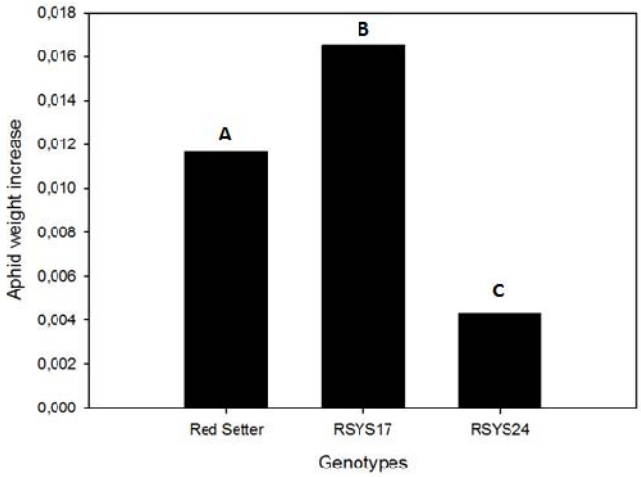


**Figure 21. Aphid weight increase after 48 hours of aphid feeding on RSYS24, RSYS17 and Red Setter plants. Letters (A, B, C) indicate three different statistical groups (One-way ANOVA).**

Aphid mortality and reproduction were also registered during this bioassay. Aphid mortality on RSYS24 plants was higher comparing to RSYS17 and Red Setter plants (data not shown). Besides, a different reproduction rate was calculated after 48 feeding hours on these transgenic plants: an average of 20 neanids was found on RSYS24, 31.7 on RSYS17 and 33 on 'Red Setter'. These two bioassays demonstrate that ProSys is able to activate responses that can affect aphid performance.

### *3.4.3 Evaluation of resistance against Botrytis cinerea*

To assess ProSys effect against fungi, a bioassays against *B. cinerea* was carried out with plants of the selected RSYS lines. Spores were used to inoculate 4 biological replicates for each genotype under investigation. As shown in figures 20 and 21 inoculated 'Red Setter' leaves were chlorotic and necrosis appeared clearly evident 24 hours *post-inoculi* becoming totally necrotic after 96 hours *post-inoculi*,

while RSYS24 and RSYS32 leaves didn't change in their color and their necrosis were much smaller. Necrosis on RSYS17 plants were also very small compared to the control, but were larger than those present on the others transgenic plants (fig. 22).

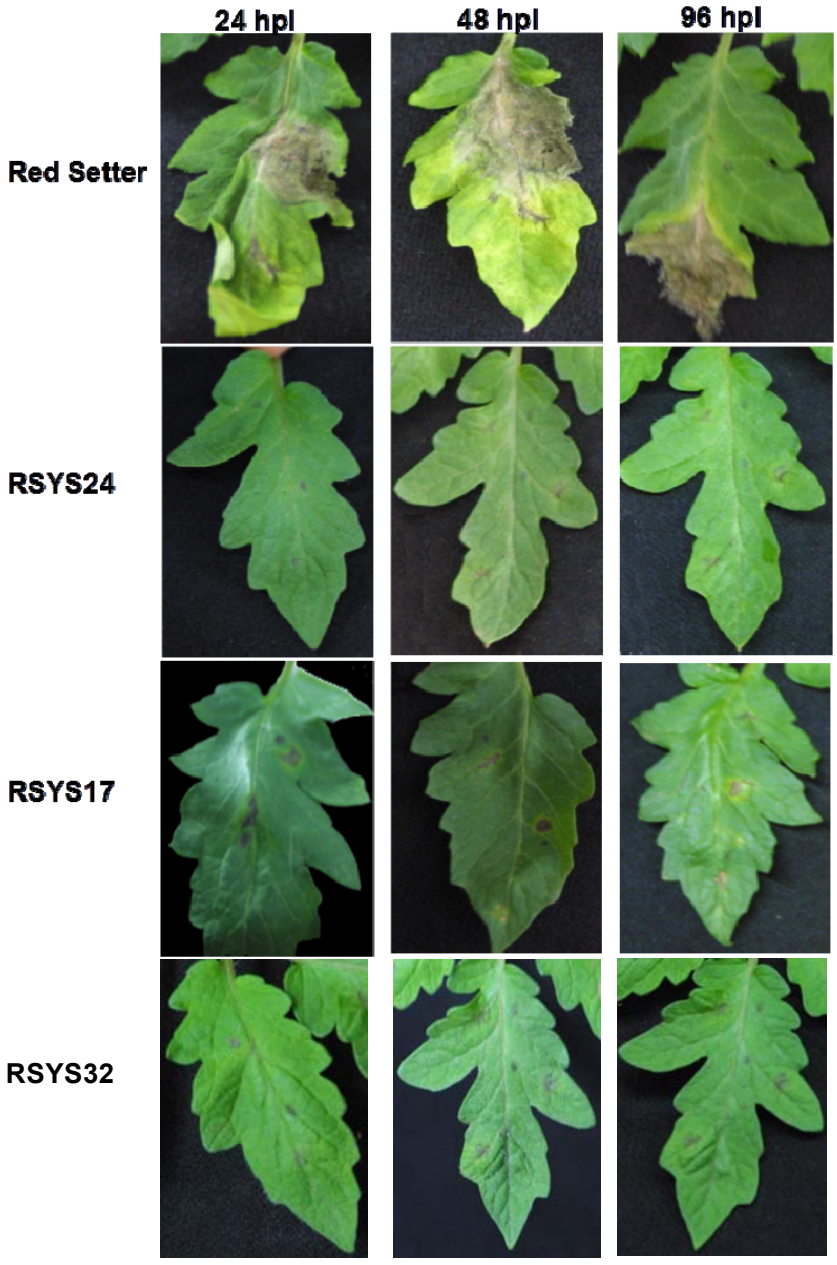


**Figure 22. Transgenic and control leaves inoculated by *B. cinerea* spores at 24, 48 and 96 hpi.**

Figure 23 displays necrosis areas measured 48 and 96 hours *post-inoculum* to support visual comparisons in fig. 20. The smallest necrosis areas were found on

RSYS24 and RSYS32 plants. Necrosis on RSYS17 had an intermediate size between Red Setter and the other transgenic lines (fig. 23).

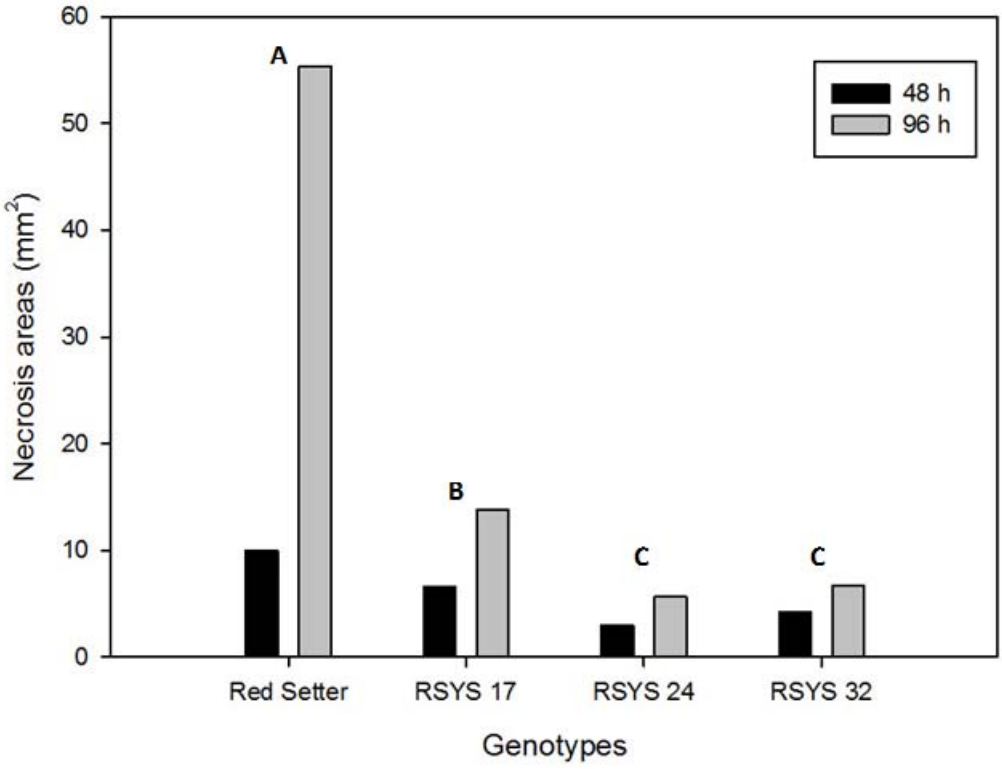


**Figure 23. Necrosis areas measured 48 and 96 hours post-inoculum of *B. cinerea* spores on RSYS24, RSYS32, RSYS17 and Red Setter plants. Letters indicate data statistical significance (One-way ANOVA; Tukey test).**

### 3.5. Local and systemic responses to *S. littoralis* mediated by ProSys

A time-course analysis was carried out positioning on each plant a *S. littoralis* third instar larva that was let to feed for one hour. Then, local and distal leaves were collected at 15 min, 30 min, 1.5, 3, 6, 9, 24 hours after larvae positioning.. Genes investigated were ProSys and InhI, chosen to analyze a gene involved in signalling and a late defence genes activated by octadecanoid pathway so associated to herbivory responses.

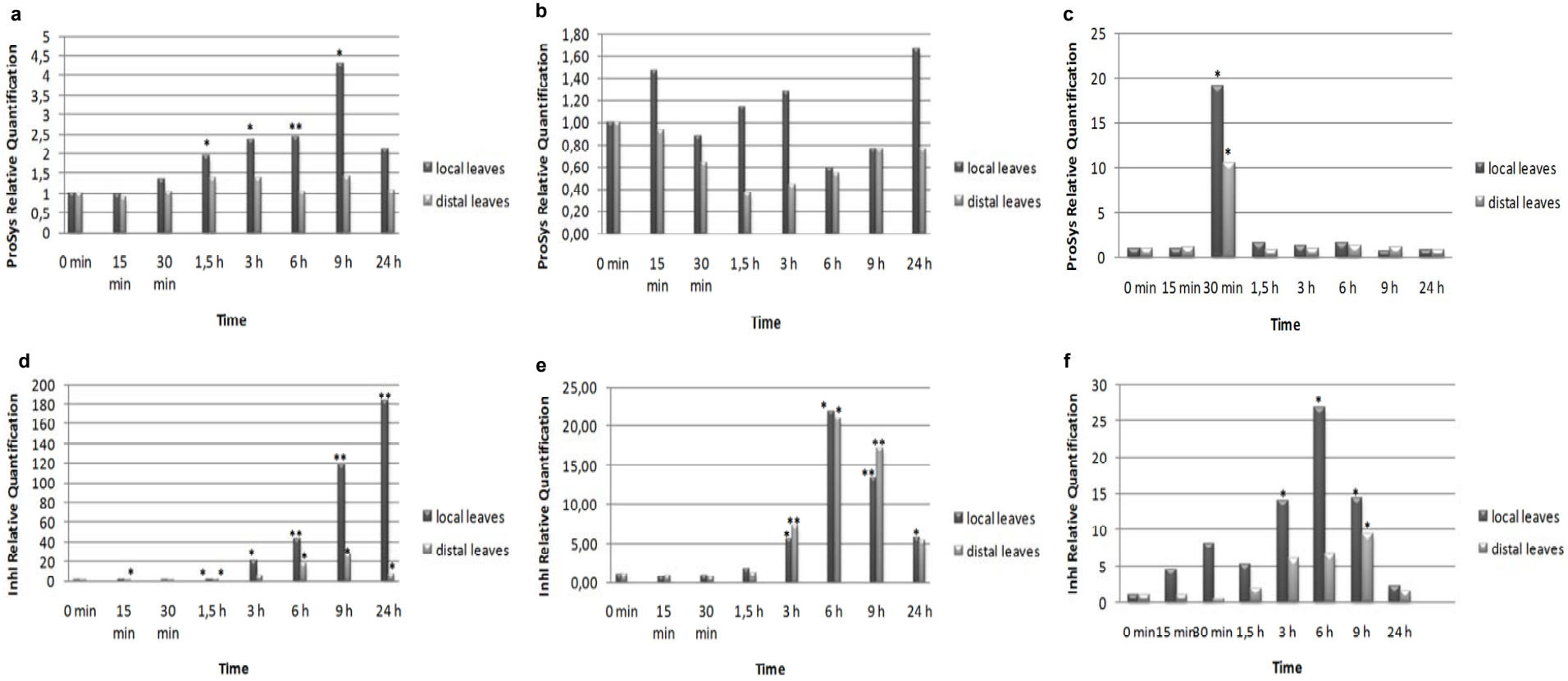


**Figure 24.** ***ProSys*** **(up) and** ***InhI*** **(down) transcripts relative quantification on local (dark grey) and distal (light grey) samples using samples at time 0 as calibrator. The analysis was carried out o Red Setter (a, d), RSYS24 (b, e) and RSYS17 (c, f) samples. On the y-axis is reported the RQ values on a linear scale. Asterisks indicate that the $2^{-\Delta\Delta Ct}$ values were significantly different (*P<0.05; **P<0.01; Student's t-test).**

This analysis was carried out on 'Red Setter', RSYS24 and RSYS17 plants to understand more on the transmission of the response signal in *wild type*, ProSys over-expressing and ProSys-suppressed tomato plants. The calibration of relative quantification was always done on samples at time 0, in this way the increase or decrease of expression could be seen. The time-course of Prosys expression in Red Setter plants showed a pick at 9 hours after larvae feeding locally, while no influence was observed systemically (fig. 24a). InhI, instead, was strongly induced close and far to the feeding site (fig. 24d) according to the widely known and reported theory that describes ProSys as the starting signal which promotes JA biosynthesis leading the transmission of the long-distance signal responsible of the plant systemic response (Sun *et al.,* 2011). On RSYS24 plants, larvae feeding didn't increase further ProSys expression neither locally nor systemically (fig. 24b). This is possibly the consequence of the very high level of Prosys expression in these plants in which Prosys dependent defences are strongly activated and the plant is pre-alerted. Surprisingly, InhI expression increased 6 hours after feeding both locally and sistemically (fig. 24e) suggesting a ProSys-indipendent PI-induction. This hypothesis was confirmed by the observations registered on RSYS17 samples in which ProSys was induced, both locally and sistemically, only 15 minutes after feeding (fig. 24c). This could be an artefact of gene silencing. Also in this sample, InhI induction initiated 6 hours after feeding both locally and sistemically and proceed similarly as registered for RSYS24 (fig. 24f). It is possible that InhI induction is generated by oligogalacturonides released from plant cell wall during larvae feeding or by larvae oral secretions in a ProSys-indipendent way.

## 3.6 Prosystemin over-expression impacts on tomato transcriptome

### *3.6.1 Genes modulated by prosystemin*

To assess changes in gene expression profiles and evaluate prosystemin impact on the whole tomato transcriptome, RSYS plants were compared with 'Red Setter' control plants by microarray analysis. A two-color labelling and a competitive hybridization of samples and controls on a 4x44K Agilent tomato chip were used. Agilent array are spotted with 43803 probes corresponding to tomato ESTs

sourced from RefSeq Release 31, Unigene Release 33, TIGR Plant TA release 5, TIGR Gene Indices Release 12. Arrays were scanned and images were firstly processed by Agilent's Feature Extraction software. This software was used to generate a quality control (QC) report for each microarray image during the extraction process. The RNA Spike-In Kit consists of two sets of positive control transcript mixtures optimized to anneal to their complementary probes on the microarray, with minimal self-hybridization and cross-hybridization. The control of the labelling and hybridization efficiency of samples was carried out referring to the Spike-In probes hybridization (Fig. 25).

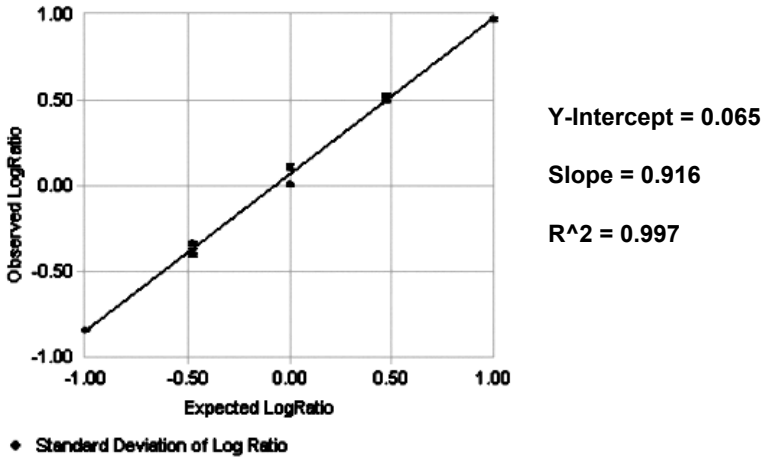


**Figure 25. Control of labelling and hybridization efficiency through the analysis of spike-In probes efficiency hybridization.**

Log ratios for cyanine 5 versus cyanine 3 signal intensities for Spike-In probes were calculated and comparing the expected log ratio and the observed one an high correlation was found ($R^2$= 0.997). The raw data and associated sample information were loaded and processed by GeneSpring® 10 (Agilent Technologies). Differentially expressed sequences were identified using a filtering by t-Student's test (p-Value < 0.05) to minimize selection of false positives. Of the significantly differentially expressed sequences, only those greater than 2-fold increase or 2-fold decrease in expression compared to the controls were used for further analysis. In order to eliminate position effect, two transgenic lines (RSYS24 and RSYS32) were analyzed jointly, them as 2 replicates. Hierarchical clustering

of differentially expressed sequences according to their expression patterns is shown in figure 26.

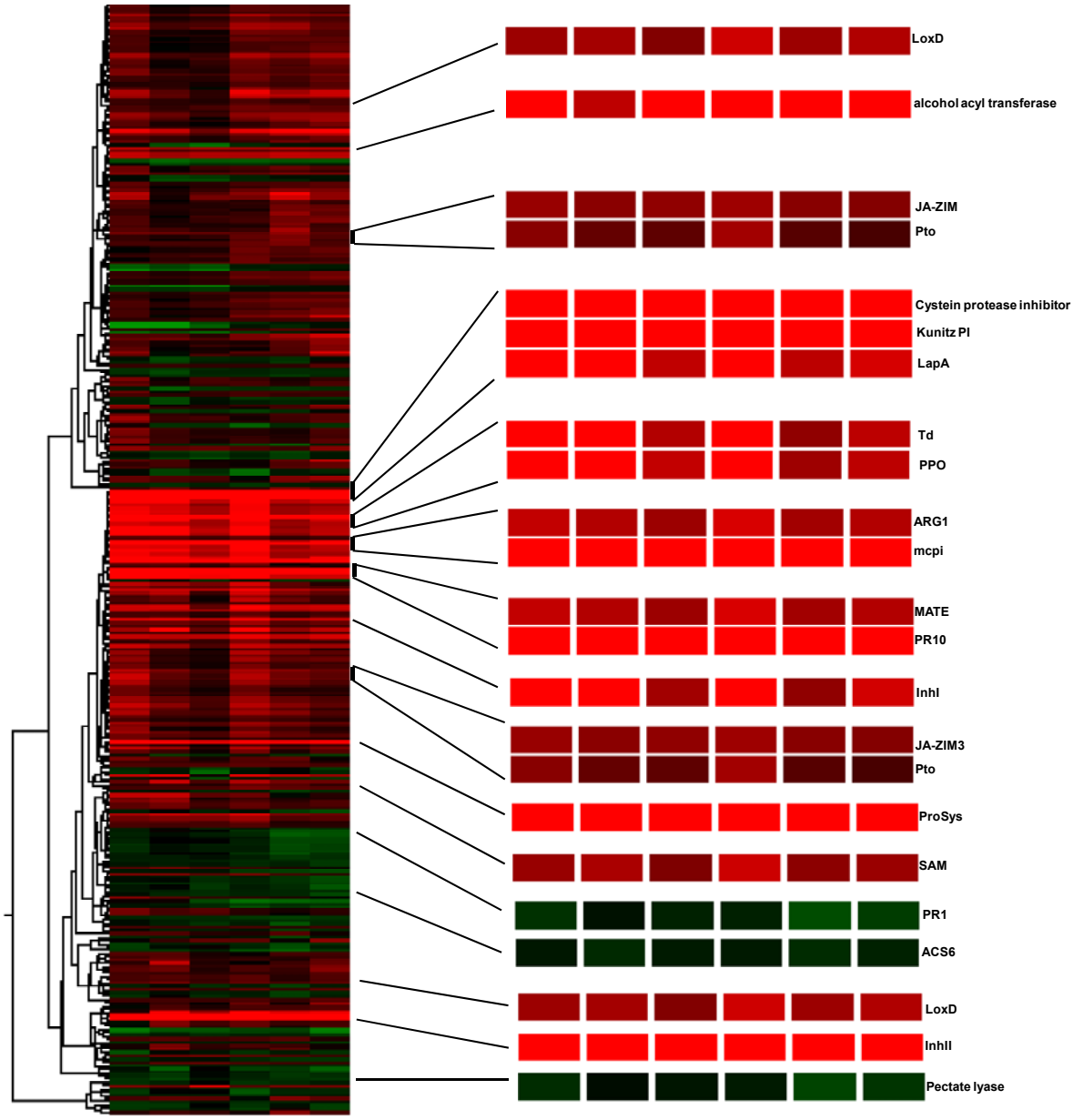


**Figure 26. Hierarchical clustering of differentially expressed sequences in RSYS samples. In red up-regulated genes are indicated, while in green the down-regulated ones.**

695 differentially expressed (fold change>2; p<0.05 T-Test) sequences were found in RSYS samples with 74% of them up-regulated and 26% down-regulated. The expression patterns of some defence genes are underlined through hierarchical cluster expansions (Fig. 26). Several JA-responsive genes, such as those coding for proteinsase inhibitors (Kunitz PI, InhI and InhII, mcpi), leucine aminopeptidase (LapA) and polyphenol oxidase (PPO) were confirmed to be induced by systemin,

as previously reported by Ryan (2000). The role of lipoxygenases (LOXs) in the octadecanoid pathway and subsequent promotion of defences against insects is well established and it has been also related to systemin-signalling pathway (Ryan, 2000). The JA-induced arginase (ARG1) and Threonine deaminase (Td) disrupt insect digestion by degrading existing amino acids necessary for insect growth (Chen et al., 2005), likely synergizing PI activity. Both genes were found to be strongly induced by ProSys over-expression. Several SA-regulated genes, such as those coding for PR proteins and Pto-responsive gene were also found to be differentially expressed. Another up-regulated gene is JA-ZIM coding for a repressor able to bind the AtMYC promoter inhibiting its enhancer activity of JA-regulated defence genes (Thines *et al.,* 2007). Two genes coding for enzymes involved in ethylene biosynthesis, such as S-adenosyl methione (SAM) and the ACC synthase (ACS6) are differentially regulated, suggesting the affection of ethylene-regulated responses in RSYS samples. The up-regulation of a drug transmembrane transporter (MATE) involved in abiotic stresses responses is also observed. ProSys over-expression also affects pectate lyase expression, which is an important player of virulence mechanisms in many soft-rotting and macerating pathogens (Jakob *et al.,* 2007). Most the 695 expressed sequences had very similar expression pattern in the six replicates used (3 replicates for RSYS24 and 3 for RSYS32). Among these, 14 sequences were validated by Real Time RT-PCR: *prosystemin* (acc. num. M84801), *leaf wound-induced proteinase inhibitor I* (acc. num. K03290), *arginase* (acc. num. AY656837), *mate efflux* (acc. num. BI933305), *LoxD* (acc. num. U37840), *subtilisin* (acc. num. TA38526_4081), *SAM* (acc. num. ES894405), *treonine deaminase* (acc. num. M61914), *osmotin* (acc. num. AY093595), *Pto locus* (acc. num. TC223474), *pathogenesis-related protein 10* (acc. num. AK329477), *kunitz protease inhibitor* (acc. num. X73986), *JA-ZIM* (acc. num. AK327683), *polyphenol oxidase* (acc. num. BI925947). Quantitative RT-PCR was carried out on RNA samples used for microarray using Red Setter samples as calibrator (fig. 27).

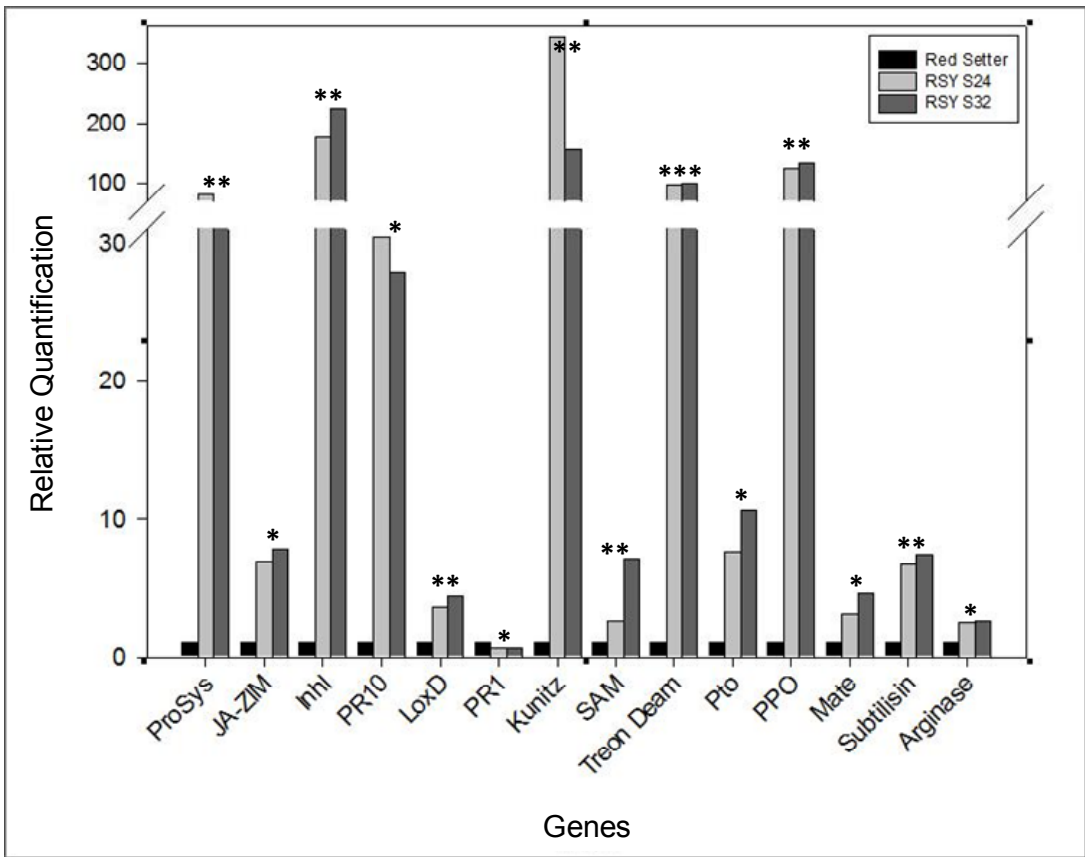


**Figure 27. The graph displays the relative quantity of target defence transcripts in the same samples used for microarray analysis. Quantities (RQ) are shown relative to the calibrator Red Setter genotype. On the y-axis is reported the RQ values on a linear scale. Asterisks indicate that the $2^{-\Delta\Delta Ct}$ values were significantly different (*P<0.05; **P<0.01; *** P<0.001; Student's t-test).**

The relative quantification of target transcripts showed that the highest up-regulated genes are those coding for PIs, observing RQ values higher than 200 for InhI and Kunitz-type PI. Threonine deaminase, PPO and PR10 were also strongly up-regulated; only PR1 was down-regulated in this gene set (Fig. 27). The expression analysis was significant at t-Student's test for all genes under investigation. Quantitative RT-PCR results were consistent and highly correlated to microarray data as shown in figure 28 in which microarray fold change and Real Time RQ values for all 14 genes under investigation were converted in a logarithmic scale ($log_2$) and compared using a xy dispersion graph. Tendency line and regression index indicate that there is an high correlation between these data.

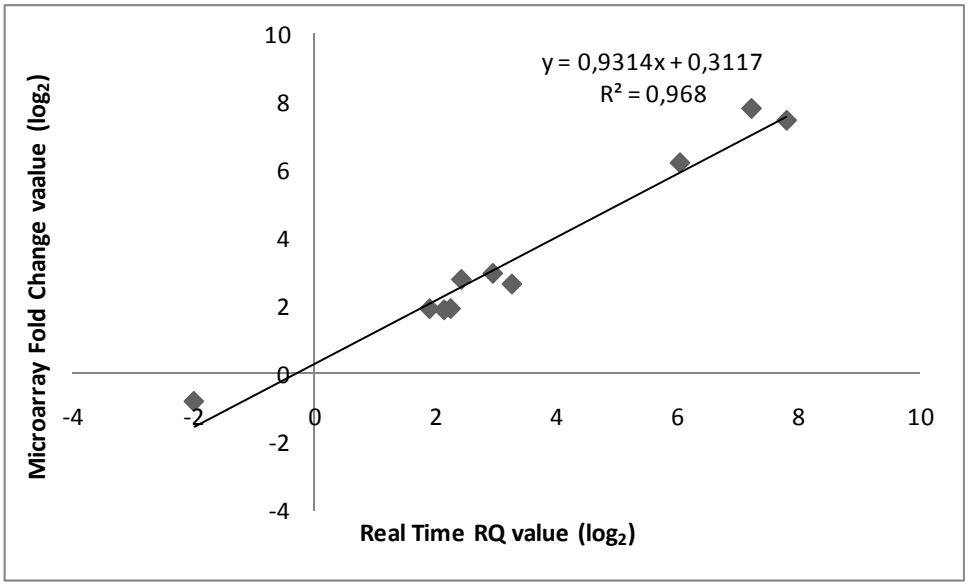


**Figure 28.The graph displays the correlation between microarray fold change and Real Time RQ values of 14 investigated defence genes using a $log_2$ scale. Tendency line and $R^2$ value are index of high correlation.**

### *3.6.2 Functional annotation of differentially expressed sequences*

Differentially expressed sequences were functional annotated with Blast2GO software (www.blast2go.org). Firstly each sequence (query) was imported into a Blast step in which the first 10 hits were collected; secondly a mapping step followed. In this step all GO terms associated to the group of hits for each query are collected. The final step represent the proper annotation step in which all GO terms mapped on the blast hits are transferred to the queries. Figure 29 summarizes the Blast2GO annotation flow chart.

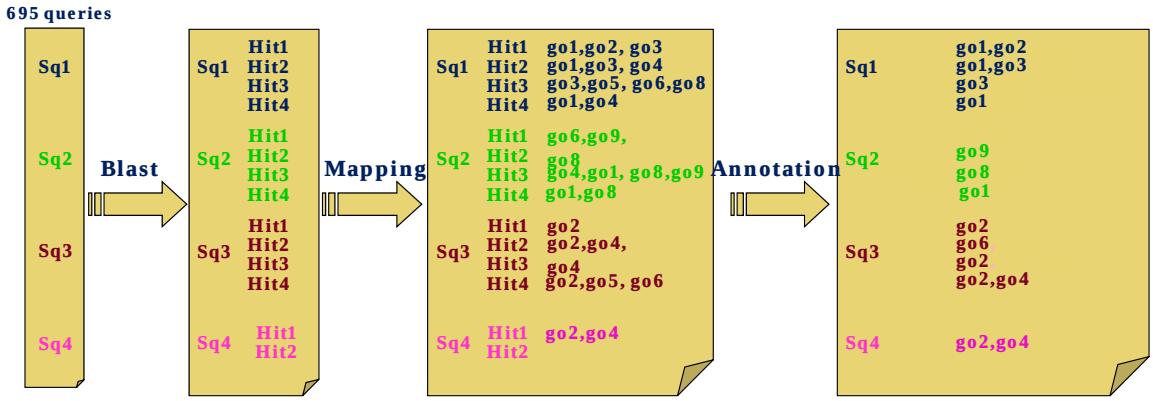


**Figure 29. The image displays the Blast2GO annotation flow chart composed by 3 steps: blast, mapping and annotation steps.**

The annotation was enriched using the InterProScan and the Annotation Augmentation tools that query different databases. The information was, then, collapsed and simplified using the GOslim tool and followed by the Kegg pathway analysis. In this way, reliable annotation for most differentially regulated genes was obtained and thanks to the Kegg pathway tool, pathways influenced by prosystemin over-expression were underlined. The pie graph in fig. 30 shows the distribution of the annotated RSYS differentially expressed sequences in classified according to the ontological domain “biological process”. As expected, large slices of the pie are related with plant defence involving the signalling, stress response and the regulation of transcription. Several sequences are specifically correlated to biotic and abiotic stresses. Still related to plant defence are also the “anatomical structure morphogenesis”, “transport”, “cell communication” categories in which several sequences were included. A big slice of the pie is also dedicated to secondary metabolism in which a wide range of toxic compounds involved not only in direct defence but also in some cases in the indirect defence are produced. Besides, the primary metabolism is also affected since many annotated sequences are associated to amino acids, lipids and carbohydrates metabolisms, cellular components organization, generation of energy, catabolic process and also to reproduction and development (Fig. 30).

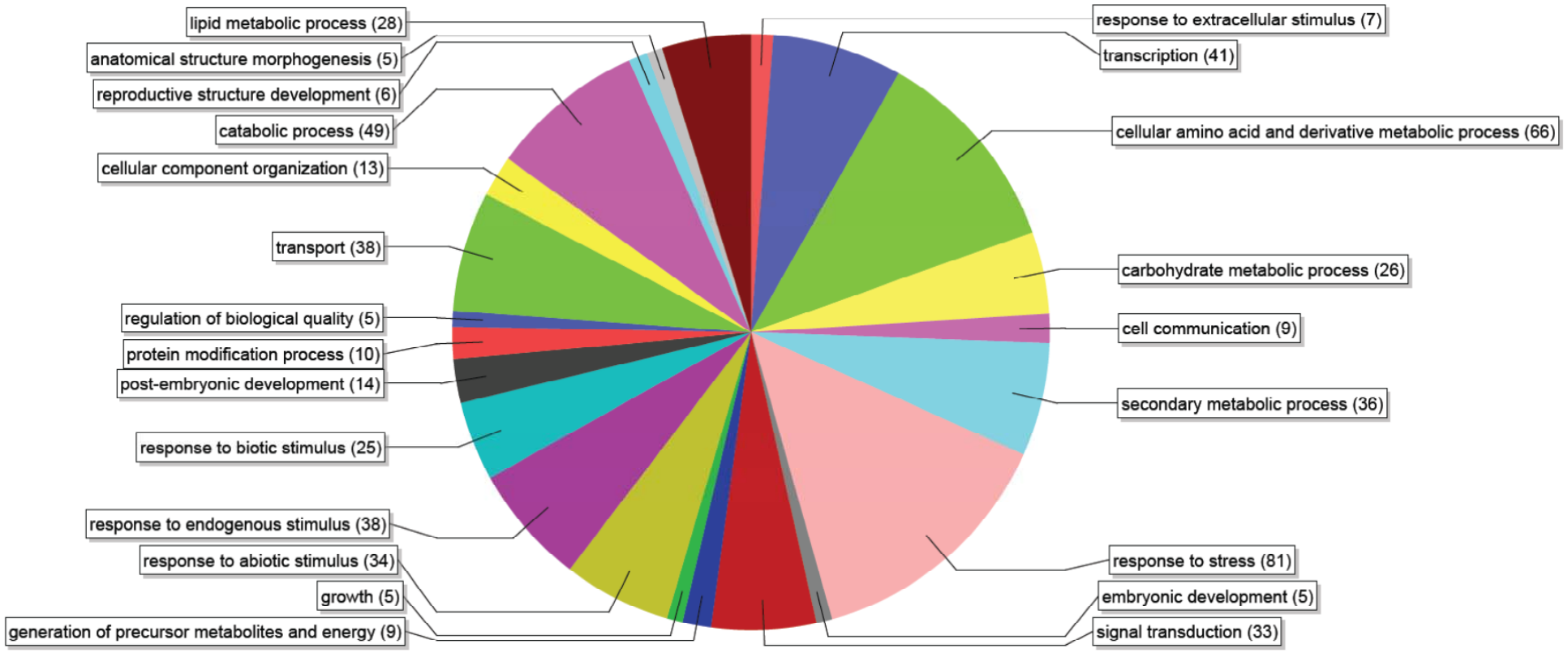


**Figure 30. The figure displays the pie chart of the distribution of differentially expressed sequences in prosystemin-overexpressing samples (RSYS) classified according to the ontological domain "biological process". Multilevel pie realized with Blast2GO software filtering by sequence cut-off=5.**

All the information obtained by the annotation analysis are summarized in table A1 (Appendix) in which the list of differentially expressed sequences with probe ID, fold change, description and functional annotations is provided. In order to explain the resistance of ProSys over-expressing plants against the biotic agents assessed (see section 3.4), several differentially expressed genes, known to be associated with this kind of responses, were underlined. An example of some herbivore-responsive genes differentially regulated in RSYS samples are listed in table 7. Most of these genes are late defence genes induced by JA and its derivatives, such as those coding for PIs, threonine deaminase, arginase, lypoxigenase and JA-ZIM domain protein, previously described in par. 3.6.1. Genes involved in signal transduction, such as calmodulin, Map kinases (MAPK3), calcium-dependent protein kinase (CDPK), purple acid phosphatise (PAP3) and LRR-domain containing receptors that are associated to responses against insects and pathogenic fungi (Wu and Baldwin, 2010) and to abiotic stress responses, are also induced by ProSys, explaining the broad spectrum action of this molecule. Besides, several transcription factors known to regulate the expression of a wide group of defence genes, such as WRKY, MYB, bZIP and AGAMOUS-like families are also differentially expressed. Allene oxide synthase (AOS) which catalyzes, in collaboration with Allene oxide cyclise (AOC), the synthesis of a key JA-precursor (OPDA), the lipase 1 involved in the beginning steps of octadecanoid pathway, eix receptor related to JA signalling and subtilisin with its inhibitor activity, are also regulated in their expression in RSYS plants. All of these genes are involved in the well characterized responses against phytophagous insects, confirming the ProSys role in the signalling and activation of responses against wounding and phytophagous insects (Ryan, 2000). Most of these genes support also the evident resistance of RSYS transgenic plants against *Botrytis cinerea* (par. 3.4.3), since necrotrophic fungi activate JA-, SA- and ET-mediated responses (Walling, 2009). So the over-expression of PIs, PPO and Lap could also be correlated to the affection of *Botrytis* performance. Extensin is an enzyme involved in cell wall organization and it is induced 7.17 times by ProSys compared to the control. Asselbergh and collaborator (2001), using a microarray approach, underlined several *Botrytis*-responsive genes and among them several JA-responsive genes coding for enzyme involved in cell-wall organization were found. Caffeoyl-O-Methyltransferase has been recently associated to responses against Botrytis

since it promotes the induced lignification after fungi penetration (Bhuiyan *et al.,* 2009). Moreover, genes involved in the phenylpropanoid pathway, which is an abundant source of anti-microbial and anti-fungal compounds and is also correlated to the lignification, are induced. The up-regulation of several SA-regulated genes, such as those coding for PR proteins and genes related to ethylene biosynthesis could explain the resistance observed against fungus and aphids. Recently, JA- and SA-defence pathways have been both linked to aphid response (Martinez de Ilarduya *et al.,* 2003; Kuśnierczyk *et al.,* 2007. A transcript coding for a protein of Gh3 family, which plays an important role in SA-mediated responses against biotrophic fungi in a Npr1-indipendent manner (Zhang *et al.,* 2008), is also up-regulated by ProSys. Some differentially expressed genes involved in abiotic stress responses are also indicated.Several differentially expressed sequences, such as those coding for annexin, UVH3, gibberellins-regulated protein 1 and gibberellins-2-oxidase (table A1), are related to responses activated by salinity supporting previous observation of ProSys involvement in salt stress tolerance (Orsini *et al.,* 2010). The gene classification proposed is not rigid, since responses against different stress factors share often common molecules. A deeper insight into transcriptional profiles determined by ProSys over-expression is provided in table A1 (appendix) in which all differentially expressed sequences are indicated.

**Table 7. ProSys-regulated genes involved in responses against herbivores and pathogenic fungi.**

| Gene name | Accession Number | GO annotation | Fold change |
|---|---|---|---|
| **Proteinase inhibitor I** | K03290 | serine-type endopeptidase inhibitor activity | 24 |
| **Proteinase inhibitor II** | K03291 | serine-type endopeptidase inhibitor activity | 92,12 |
| **Metallocarboxypeptidase inhibitor precursor** | X59285 | serine-type endopeptidase inhibitor activity | 48,34 |
| **Kunitz-type protease inhibitor** | X73986 | endopeptidase inhibitor activity<br>response to bacterium | 119,8 |
| **Leucine aminopeptidase** | U50152 | defence response to insect | 82,2 |
| **Treonine deaminase** | M61914 | regulation of transcription | 17,87 |
| **Arginase** | AF146690 | polyamine metabolic process | 7,68 |
| **Lipase 1** | BG631546 | hydrolase activity<br>phospholipase activity | 2,05 |
| **Allene oxide synthase** | AJ271093 | response to wounding<br>oxylipin metabolic process<br>response to jasmonic acid stimulus | 9 |
| **Lipoxygenase (loxD)** | U37840 | lipoxygenase activity<br>Jasmonic acid mediated signalling | 3,26 |
| **JA-ZIM domain protein 1** | BT013158 | Jasmonic acid mediated signalling | 24,88 |
| **JA-ZIM domain protein 3** | BI209348 | Jasmonic acid mediated signalling | 2,42 |
| **Subtilisin-like protease** | TC239872 | Jasmonic acid mediated signalling | 9,55 |
| **Polyphenol oxidase** | BI206363 | defence response<br>response to fungi | 4,34 |
| **SDM1** | AW094391 | response to herbivore<br>glucosinolate catabolic process | 2,2 |
| **Alpha/beta-hydrolase domain-containing protein (det2)** | AJ786362 | oxidoreductase activity<br>hydrolase activity | 2,97 |
| **Leucine rich protein** | DB684300 | response to fungus<br>transmembrane receptor protein<br>tyrosine kinase signaling pathway | 2,9 |
| **Phenylalanine ammonia-lyase** | BI933291 | phenylpropanoid biosynthetic process<br>response to wounding<br>response to oxidative stress | 3,68 |
| **4-coumarate:CoA ligase 1** | AK328438 | phenylpropanoid biosynthetic process<br>response to wounding<br>response to oxidative stress | 2,78 |

| | | | |
|---|---|---|---|
| **4-coumarate:CoA ligase 2** | AK323545 | phenylpropanoid biosynthetic process | 2,24 |
| **cinnamate-4-hydroxylase** | BE431646 | phenylpropanoid biosynthetic process<br>response to wounding<br>response to oxidative stress | 2,15 |
| **Caffeoyl- o-methyltransferase** | AK322239 | response to cadmium ion<br>coumarin biosyntheti process<br>lignin biosynthetic process | 3,18 |
| **Apoptosis-related protein** | AK319191 | apoptosis<br>defense response to fungus<br>response to hydrogen peroxide<br>calmodulin binding | 2,31 |
| **Calmodulin** | AK324373 | calcium-mediated signalin | 3,22 |
| **Osmotin** | AY093595 | defence response to bacterium<br>defence response to fungus | 2,16 |
| **PR-10 type pathogenesis-related protein** | AK329477 | systemic acquired resistance<br>response to cold, UV light, cadmium | 7,25 |
| **PR-1 pathogenesis-related protein** | X71592 | systemic acquired resistance<br>response to salicilic acid | -3,98 |
| **PR-2 pathogenesis-related protein** | AK327264 | systemic acquired resistance<br>response to salicilic acid | 2,6 |
| **Chitinase** | TA36496_4081 | response to other organisms<br>cell wall macromolecule catabolic process | -4,22 |
| **Beta-glucosidase 17** | AK320730 | hydrolase activity, hydrolyzing O-glycosyl compounds<br>lignin biosynthetic process | 2,37 |
| **Gh3 family protein** | BI205334 | response to auxin stimulus<br>response to fungi | 2,03 |
| **1-aminocyclopropane-1-carboxylate oxidase** | X04792 | ethylene biosynthetic process | 3,22 |
| **ethylene-responsive transcription factor ERF** | EG553122 | regulation of transcription, DNA-dependent<br>response to fungi<br>response to chitin | 2,75 |
| **S-adenosyl methionine** | AK322239 | ethylene biosynthetic process | 3,18 |
| **BCL-2-associated athanogene 6** | AK319191 | response to $H_2O_2$<br>apoptosis | 2,31 |
| **Expansin** | AF096776 | plant cell-type wall organization | -2,1 |
| **Cell-wall invertase** | AB004558 | plant cell-type wall organization | -2,14 |

| | | | |
|---|---|---|---|
| **MAP3K delta-1 protein kinase** | BI924417 | protein serine/threonine kinase activity<br>response to salt stress | 2,43 |
| **Calcium-dependent protein kinase (CDPK)** | BI206321 | serine/threonine kinase activity<br>calcium binding | 5,79 |
| **Purple acid phosphatase 3 (PAP3)** | AW738593 | serine/threonine kinase activity | 2,23 |
| **serine acetyltransferase** | DB683870 | response to sulfate starvation<br>response to cold | -2,34 |
| **WRKY DNA-binding protein 65** | AY157061 | sequence-specific DNA binding transcription factor activity | 2,34 |
| **WRKY DNA-binding protein 40** | TA37286_4081 | sequence-specific DNA binding transcription factor activity | 5,85 |
| **bZIP transcription factor** | AK247747 | transcription factor activity<br>regulation of transcription | -4,97 |
| **WRKY transcription factor 1** | AI485880 | cellular response to phosphate starvation<br>regulation of transcription<br>response to chitin | 6,48 |
| **MYB-related transcription factor** | BI931087 | regulation of protein localization<br>regulation of transcription<br>trichome patterning | 12,99 |
| **AGAMOUS-like 20** | BF098196 | regulation of transcription<br>response to cold | 3,48 |
| **Ap2-erf domain containing transcription factor** | AK321000 | Jasmonic acid and ethylene-mediated systemic resistance<br>regulation of transcription | 2,34 |
| **Eix receptor 2** | TA53354_4081 | receptor activity<br>signal trasduction | 2,7 |
| **Gh3 family protein** | BI205334 | response to auxin stimulus<br>response to fungi | 2,03 |
| **EF Tu receptor** | DB684300 | plant-type hypersensitive response<br>LRR serine/threonine kinase activity<br>detection of bacterium | 2,09 |
| **Monoterpene synthase 1 (MTS1)** | AY840091 | sesquiterpene synthase activity<br>terpenoid biosynthesis | 5,39 |
| **GCR2-like 1** | AK321920 | response to salt stress | 3,85 |
| **chaperonine 60-alpha** | BT013144 | chloroplast organization<br>protein folding | 2 |
| **Low temperature and salt responsive element** | AK323093 | abiotic stress response | 2,13 |

## 3.7 Defence genes expression analysis after plant-aphid and plant-pathogen interactions

The expression analysis of genes involved in responses against different pests in time-course experiments is a very powerful tool to shed more light on ProSys specific activity in the signalling pathway. To this aim, genes reported to be elicited by aphids and fungi were analyzed after induction.

### *3.7.1 Aphid-induced genes expression analysis*

In order to underline the relationship between ProSys and key-genes involved in responses against piercing-sucking insects, a time-course analysis of defence gene expression after aphid infestation was performed on Red Setter plants. The aim was to compare aphids sucking activity on the expression profiles of selected candidate genes with the expression profiles determined by ProSys over-expression. In these bioassays each plant was infested with 15 aphids that were positioned on an enclosed area collecting local and distal leaves after 24, 48 and 96 hours after infestation. Genes to investigate (table 8) were selected from microarray data of RSYS plants, from microarray data on *S. lycopersicum* cv. "Microtom" infested by aphids, available in the lab, and from previously published data (Kusnierczyc *et al.,* 2007; 2008).

| Name | Genebank_Accession | Fold change |
|---|---|---|
| Lipoxygenase D (LoxD) | U37840 | 3,727 |
| Wound-induced proteinase inhibitor I | K03290 | 24 |
| Wound-induced proteinase inhibitor II | K03291 | 92,12 |
| Kunitz-type proteinase inhibitor | X73986 | 119,8 |
| Leucine aminopeptidase | U50152 | 82,2 |
| Threonine deaminase | M61914 | 17,87 |
| Pathogenesis-related protein 1 | X71592 | -3,96 |
| WRKY40 | AK325041 | 2,36 |

**Table 8. Aphid-induced defence genes chosen for the expression analysis. Genes names, Genebank accession numbers and expression values from RSYS microarray are listed.**

A time-course relative expression analysis of the selected genes was carried out on Red Setter plants (fig. 31).

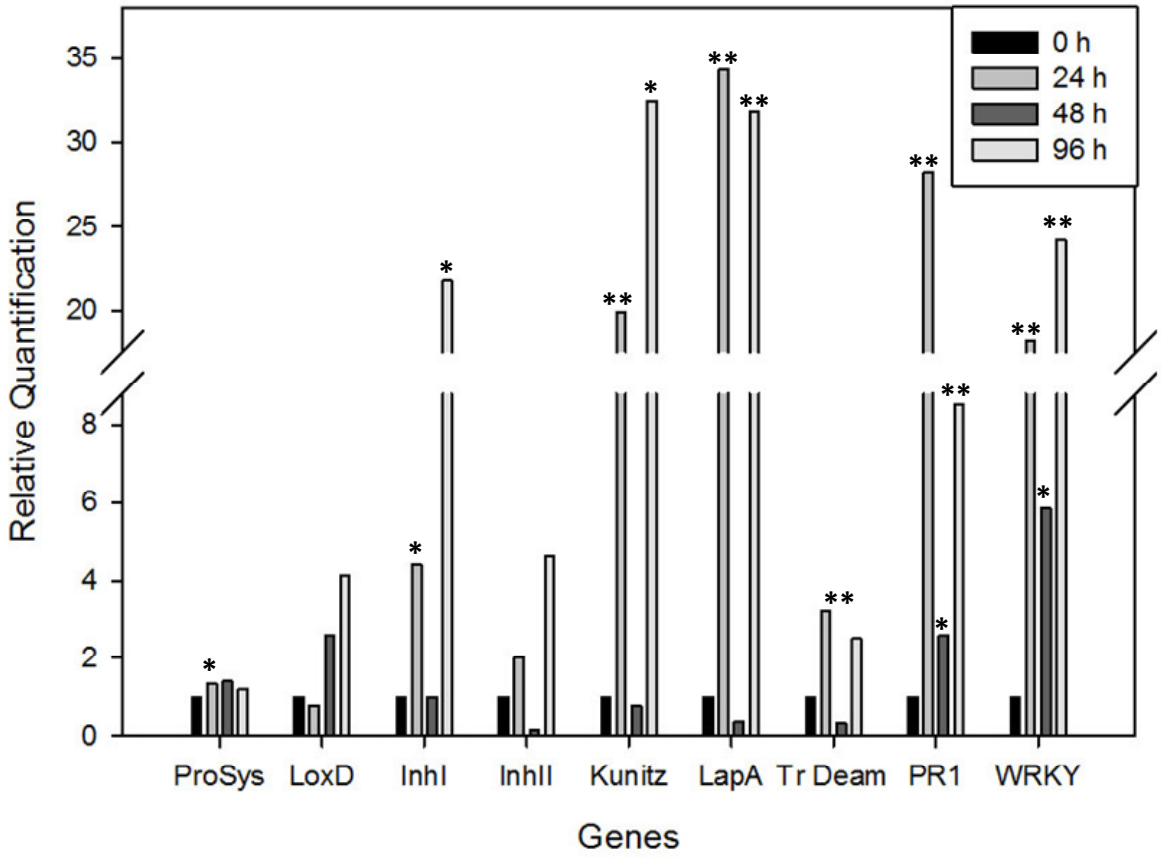


**Figure 31. Time course of defence genes expression after *M. euphorbiae* infestation on 'Red Setter' plants. The graph displays the relative quantity of defence transcripts in Red Setter plants 24, 48 and 96 hours after infestation. Quantities (RQ) are shown relative to the calibrator Red Setter 0 h. On the y-axis is reported the RQ values on a linear scale. Asterisks indicate statistical significance by t-Student's test(*p<0.05; **p<0.01; ***p<0.001)**

This analysis demonstrated that aphid feeding on tomato plants induces several JA-responsive genes: *LoxD* that is an early gene of the octadecanoid pathway, *LapA* and threonine deaminase, late defence genes coding for different classes of proteinase inhibitor. Moreover, SA-regulated genes such as *PR1* and *WRKY* are also strongly induced. All these genes are affected in their expression by *ProSys*, as indicated in table 8. Fig. 32 shows the relative expression analysis of these genes on RSYS24 and RSYS17 lines compared to the control "Red Setter".

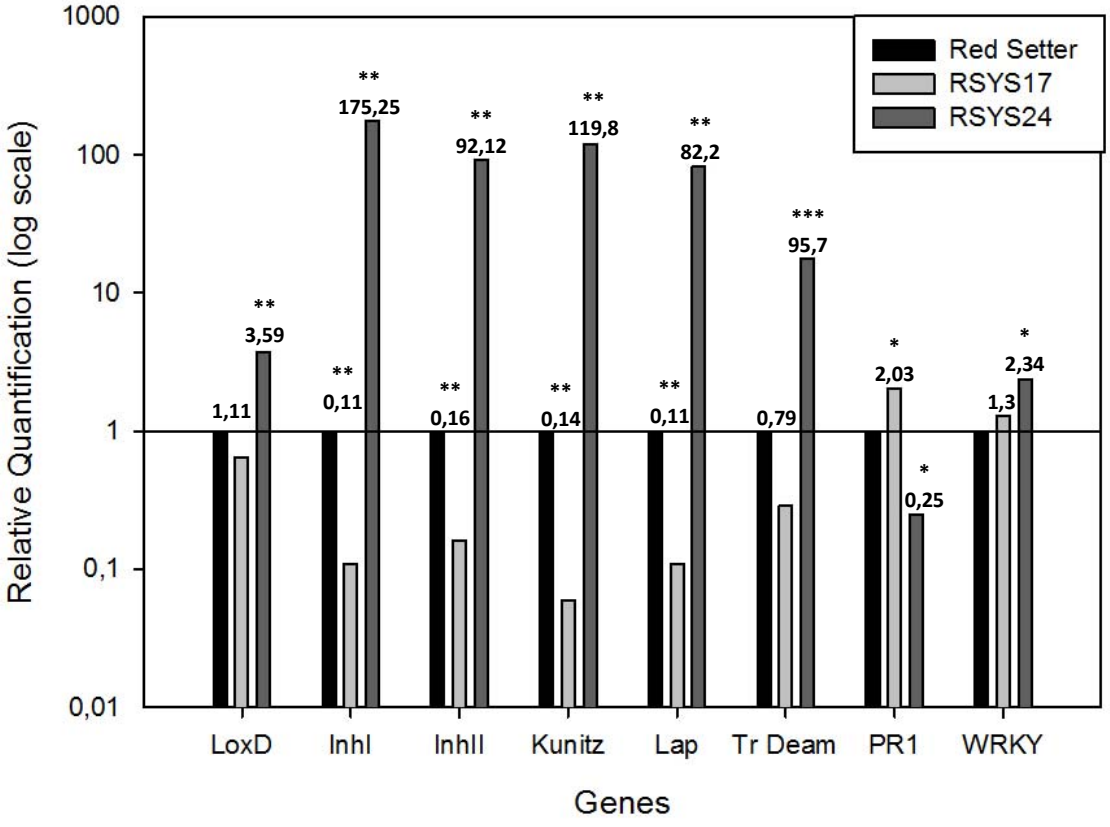


**Figure 32. Relative expression analysis by Real Time RT-PCR of genes involved in responses to *M. euphorbiae*. The graph displays the relative quantity of defence transcripts in RSYS24 and RSYS17 lines compared to "Red Setter" plants. On the y-axis is reported the RQ values on a logarithmic scale. Asterisks indicate statistical significance by t-Student's test(*p<0.05; **p<0.01; ***p<0.001)**

ProSys over-expression induces genes involved in JA- and SA-regulated defence pathways (fig. 32). ProSys over-expression induces genes involved in JA- and SA-regulated defence pathways (fig. 32). This finding leads to the conclusion that ProSys over-expression activates signaling pathways involved in signal response to aphid feeding  In the co-suppressed lines all these genes are down-regulated, except PR1, suggesting that ProSys regulates many but not all pathways that are involved in aphid response. Interestingly, PR1 is down-regulated in RSYS samples implying that SA-dependent gene is not essential to increase resistance to aphids in RSYS transgenic lines.

### *3.7.2 Genes modulated by B. cinerea*

To analyze fungi-responsive genes regulated by ProSys we used the same approach adopted for aphids. A gene expression time-course analysis on Red Setter plants was performed on 3 biological replicates for each time point harvesting samples at 48 and 96 hours *post-inoculi* (hpi). Microarray data on tomato inoculated by *Botrytis* spores (Asselbergh et al, 2001) helped to underline fungi-related responses in the microarray dataset produced (table A1) and to extend the analysis on some new *Botrytis* specific responsive-genes. Among them, 7 plus ProSys were chosen for this analysis (table 9).

| Name | Genebank_Accession | Fold change |
|---|---|---|
| Extensin | X55688 | 2,42 |
| Osmotin | AY093595 | 2,17 |
| Arginase | AK321112 | 17,49 |
| Wound-induced proteinase inhibitor I (InhI) | K03290 | 24 |
| Lipoxygenase A (LoxA) | SGN-U143303 | - |
| Pti5 | U89256 | - |
| Miraculin | SGN-U144553 | - |

**Table 9. The table indicates Botrytis-induced defence genes chosen for the expression analysis providing name, Genebank accession numbers and expression values from RSYS microarray. The last 3 genes were added to the analysis referring to Asselbergh and collaborators' study (Asselbergh *et al.*, 2001).**

Figure 33 shows the time-course analysis on Red Setter plants for the genes under investigation. The expression analysis of the selected genes showed that the fungus inoculums induces mainly SA-regulated genes in the first 48 h post-inoculum, but at 96 h post-inoculum a significant up-regulation of the wound-induced proteinase inhibitor (*InhI*) was also observed (fig. 33).

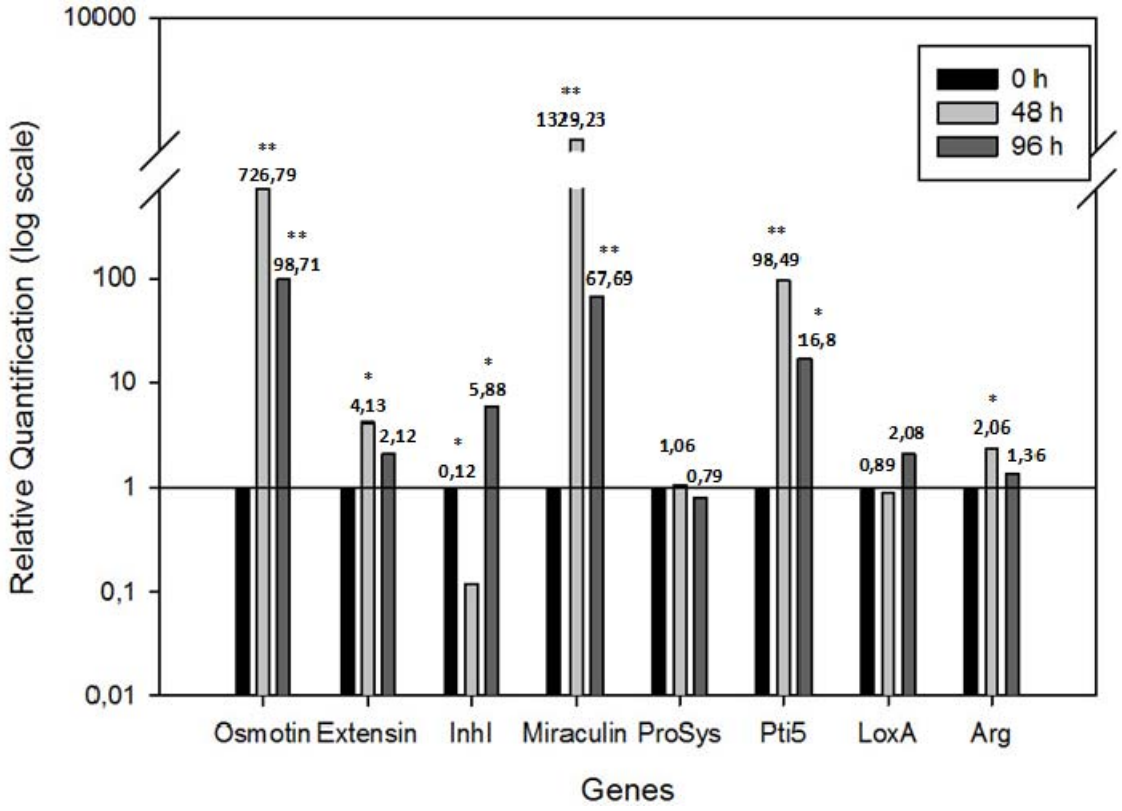


**Figure 33. Time course of defence genes expression after *B. cinerea* inoculi on Red Setter plants. The graph displays the relative quantity of defence transcripts in Red Setter plants 48 and 96 hours post-inoculum. Quantities (RQ) are shown relative to the calibrator Red Setter 0 h. On the y-axis is reported the RQ values on a logaritmic scale. Asterisks indicate that the $2^{-\Delta\Delta Ct}$ values were significantly different (*P<0.05; **P<0.01; Student's t-test).**

In order to investigate a possible ProSys influence on the expression of these *Botrytis*-induced genes, their relative expression was evaluated on un-infested RSYS24 and RSYS17 genotypes using 'Red Setter' as calibrator (fig. 34).

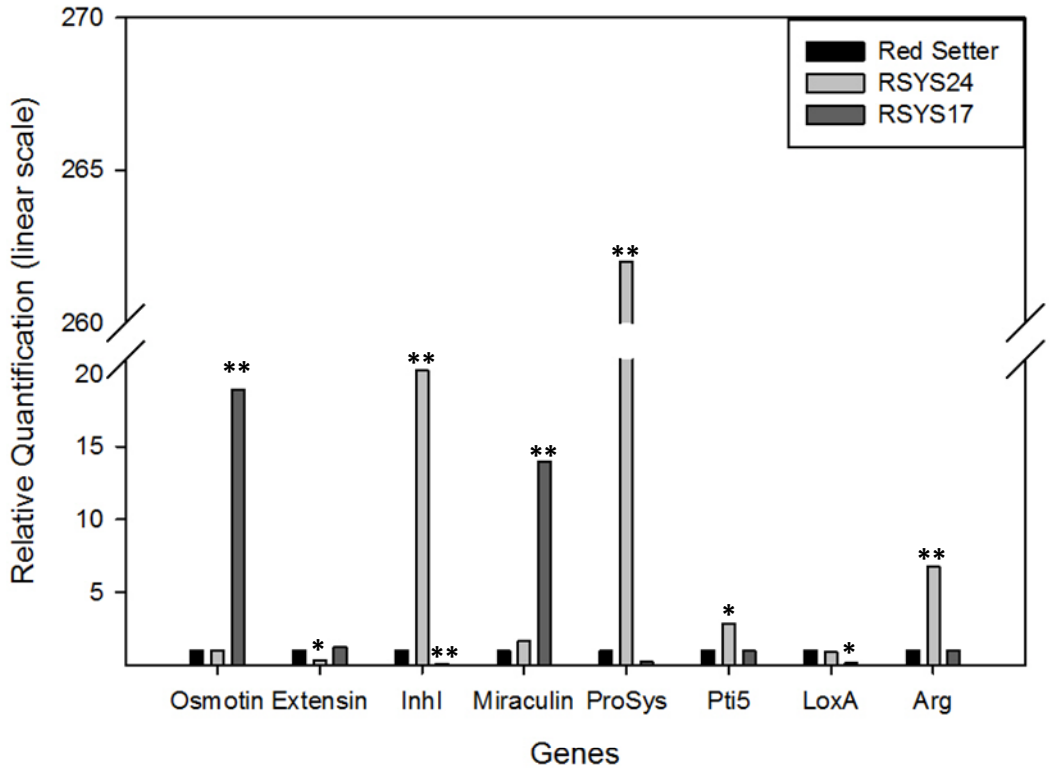


**Figure 34. Relative expression of defence genes involved in responses against fungi by Real Time RT-PCR. The graph displays the relative quantity of target defence transcripts in RSYS and control plants without Botrytis inoculums. Quantities (RQ) are shown relative to the calibrator Red Setter genotype. On the y-axis is reported the RQ values on a linear scale. Asterisks indicate that the $2^{-\Delta\Delta Ct}$ values were significantly different (*P<0.05; **P<0.01; Student's t-test).**

The main actors of *Botrytis*-induced responses appeared to be osmotin, extensin, InhI, arginase, miraculin and *Pti5*. Comparing these data with the expression analysis before *Botrytis* inoculi (fig. 34), only *InhI* and *Pti5* were significant up-regulated in RSYS24, while osmotin and miraculin resulted up-regulated in RSYS17. So the strong and the intermediate resistance of RSYS24 and RSYS17 (par.3.4.3), respectively, against this pathogenic fungus could be due to different defence pathways. In RSYS24 a ProSys- and JA- but also SA-mediated pathways could explain the resistance against *B. cinerea*. RSYS17 was not as strongly resistant as RSYS24 and RSYS32, but necrosis were significantly smaller than the control (par.3.4.3). These observations, in addition to the up-regulation of osmotin and miraculin (fig. 34), suggest the promotion of SA-mediated responses in RSYS17 line in which JA-mediated responses are suppressed.

### 3.8 Functional analysis: shifting to Arabidopsis interactome

In order to get a deeper and more defined overview of the *Prosys* signaling pathway and to extend the functional analysis also to the study of predictable protein-protein interactions (PPIs) in RSYS plants, each tomato ESTs was assigned to its nearest homologous gene from Arabidopsis. Arabidopsis is, in fact, the model plant species, widely studied and reported for which a very deep and detailed knowledge is available. It is often used as the unique model reference for plant kingdom by many bioinformatic tools, such as the Paintomics tool (www.paintomics.org), used for the integration and visualization of transcriptomic data. Besides, in order to insert the differentially expressed sequences found in RSYS samples in a wider study of protein-protein interactions (PPIs), a protein interactome was needed and it was not available for tomato. To these aims, the 695 differentially expressed tomato ESTs were converted in their Arabidopsis correspondent proteins. Fig. 35 shows the flow chart of the functional analysis performed.

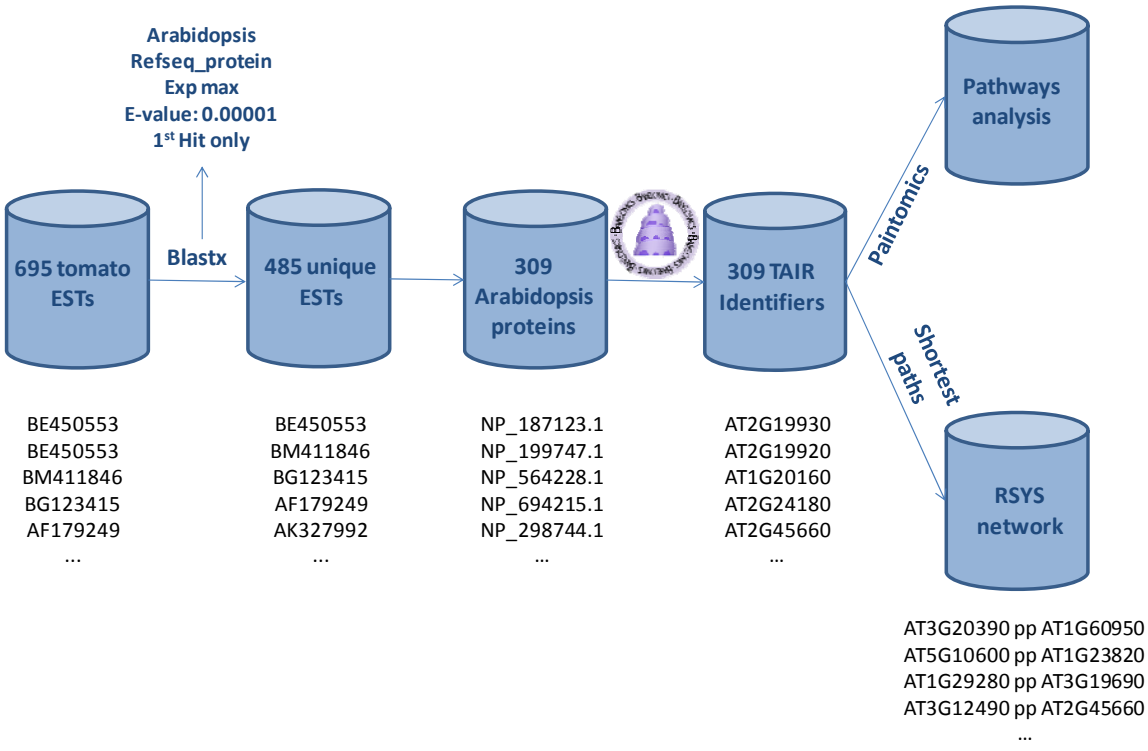


**Figure 35. The figure displays the flow chart used to perform the analysis of pathway influenced by ProSys over-expression and the PPIs network using correspondent Arabidopsis TAIR identifiers.**

The nearest homologous genes from Arabidopsis were obtained through blastx analysis using the Arabidopsis RefSeq database as reference, applying an e-value filter (Exp Max=$10^{-5}$) and collecting only first hit for each query. From the starting 695 differentially expressed sequences, a list of 309 Arabidopsis proteins was obtained. To get the TAIR identifiers for these proteins, the identifier converter available at Babelomics 4.2 website (www.babelomics.org) was used. The protein list obtained in this way was ready to be used for the Paintomics and PPIs analysis.

Analysis of defence pathways influenced by prosystemin over-expression was carried out using the Paintomics tool (www.paintomics.org) developed at CIPF (Centro de Investigation Principe Felipe, Valencia), suitable for the integration and visualization of transcriptomic data. Some stress related pathways were selected for further investigations: attention was focused on phenylpropanoid biosynthesis and the related phenylalanine and flavonoid metabolisms, plant-pathogen interactions and the arginine and proline biosynthesis. Several ProSys regulated-genes coding for enzymes located in very crucial locations of these defence-related pathways are underlined in fig. 36-40. Red boxes represent up-regulated proteins while green boxes represent down-regulated ones. In the images, enzymes corresponding to the regulated genes in RSYS plants are indicated by the Arabidopsis TAIR identifiers. Table 10 indicates a list of the genes involved in these pathways providing GeneBank accession of the tomato ESTs, the Arabidopsis corresponding TAIR code that is shown in the pathway images, the name and the microarray fold change. Some enzymes are repeated into the pathways since they can code several chemical reactions based on the availability of their substrates and the feedback control of the pathway producing different compounds.

**Table 10. The table displays some ProSys-regulated genes related to stress response pathways.**

| Genebank_Accession | TAIR_Accession | Name | Fold change |
|---|---|---|---|
| AK321203 | AT4G17830 | Peptidase M20/M25/M40 family protein | 25,67 |
| AW625830 | AT5G48930 | hydroxycinnamoyl-Coenzyme A shikimate/quinate hydroxycinnamoyltransferase (HCT) | 24,78 |
| AK323579 | AT3G21240 | coumarate:CoA ligase 2 (4CL) | 21,84 |
| DB684300 | AT5G20480 | leucine-rich repeat receptor kinase (LRR-RLK) | 20,93 |
| AK324517 | AT5G37780 | Calmodulin 1 | 20,06 |
| AK327977 | AT4G08900 | Arginase | 17,49 |
| AK320918 | AT4G36220 | ferulate 5-hydroxylase (F5H) | 10,79 |
| TA48362_4081 | AT5G53130 | Cyclic nucleotide gated channel family | 5,192 |
| BI924804 | AT5G53120 | Spermidine synthase 3 | 4,24 |
| BI933291 | AT3G10340 | Phenylalanine ammonia lyase 4 | 3,6 |
| AK328438 | AT1G51680 | 4-coumarate--CoA ligase 1 | 2,76 |
| EF550528 | AT3G25570 | S-adenosylmethionine decarboxylase | 2,62 |
| BI209348 | AT3G17860 | Jasmonate ZIM-domain protein 3 | 2,429 |
| BP892917 | AT2G40890 | coumarate 3-hydroxylase (C3H) | 2,2 |
| AK323147 | AT2G40890 | cytochrome P450 98A3 | 2,16 |
| BE450553 | AT2G30490 | Cinnamate-4-hydroxylase | -2,03 |
| AK326775 | AT3G48000 | (NAD+) aldehyde dehydrogenase | -2,2 |

The phenylpropanoid biosynthesis produces a large family of compounds showing strong anti-microbial and anti-fungal activities (Dixon *et al.,* 2002). As shown in fig. 36, the activity of several enzymes involved in this pathway is induced. The up-regulated genes coding for phenylalanine ammonia lyase 4 (AT3G10340) and coumarate:CoA ligase 2 (AT3G21240) promote coumarin accumulation. These compounds are induced under several stress conditions, have anti-microbial and anti-oxidative activities and appear to play an important role in disease resistance. Besides, one of the firstly discovered coumarin, scopoletin, has been associated to plant responses against viruses (Chong *et al.,* 2002). The up-regulation of Cinnamate-4-hydroxylase (AT2G30490) pushes forward the reactions involving coumaric and caffeic acids catalyzed by hydroxycinnamoyl-Coenzyme A shikimate/quinate hydroxycinnamoyltransferase (HCT) (AT5G48930), the above-mentioned coumarate:CoA ligase 2 (AT3G21240) and cytochrome P450 98A3 (AT2G40890). Genes coding for ferulate 5-hydroxylase (F5H) and coumarate 3-hydroxylase (C3H) are also induced. These genes are responsible of monolignols biosynthesis that are transported to the cell wall through an unclear mechanism where they are involved in peroxidase- and laccase-catalyzed reactions polymerizing lignin (Naoumkina *et al.,* 2010). The related phenylalanine metabolism is also regulated in some enzymes active at the initial steps (fig. 37). The accumulation of trans-Cinnamate which represents a central compound is

promoted since the up-regulation of phenylalanine ammonia lyase 4 and the down-regulation of cinnamate-4-hydroxylase (AT2G30490). The accumulation of capsaicin is induced by coumarate:CoA ligase 2 (AT3G21240) and cytochrome P450 98A3 (AT2G40890). Capsaicin is included in the family of capsaicinoids that protect *Capsicum chacoense* seeds from *Fusarium spp.* infection. Interestingly, these compounds are found only within the fruit of *Capsicum* species and their concentrations increase during fruit ripening, so their function seems to be restricted to their anti-microbial and anti-fungal activities (Tewksbury *et al.,* 2008). Flavonoids are included in the large family of phenylpropanoid compounds and they have been used for centuries to treat microbial diseases. They explicit their anti-microbial and anti-fungal properties acting on the functionality of biological membranes (Tamba *et al.,* 2007). In the flavonoid biosynthesis (fig. 38) p-Coumaroyl-CoA, mainly produced by the phenylpropanoid pathway, can be converted directly in caffeoyl-CoA or indirectly through two different acid intermediates: shikimic and quinic acids. All these ways include cytochrome P450 98A3 (AT2G40890) and hydroxycinnamoyl-Coenzyme A shikimate/quinate hydroxycinnamoyltransferase (HCT) (AT5G48930), both coded by ProSys-induced genes. Caffeoyl-CoA is a central compound of all above-mentioned pathways since it is a precursor of many compounds of phenylpropanoid family. Figure 39 shows a scheme about the plant-pathogen interaction in which a LRR-RLK receptor (AT5G20480) and a $Ca^{2+}$ channel (AT5G53130) located on the cell surface are coded by RSYS up-regulated genes. The signal transduction initiated by $Ca^{2+}$ influxes is then transmitted by calmodulin 1 (AT5G37780), that is linked to ROS production and the following hypersensitive response, cell wall fortification and stomatal closure. These events characterize the PAMP-triggered immunity, so the initial phase of plant-pathogen interaction based on the ZigZag model from Jones and Dangle (2006). Besides, the gene coding for JA-ZIM 3 (AT3G17860) is induced. This gene is part of a wide family of JA-signalling repressor, previously cited in par. 3.4.1. The up-regulation of this gene fits into the context of defense gene regulation in response to pathogens, mainly necrotrophic, controlled by JA. Fig. 40 shows arginine and proline biosynthesis in which genes coding for several enzymes at the beginning and ending steps of the pathway are regulated by ProSys over-expression.

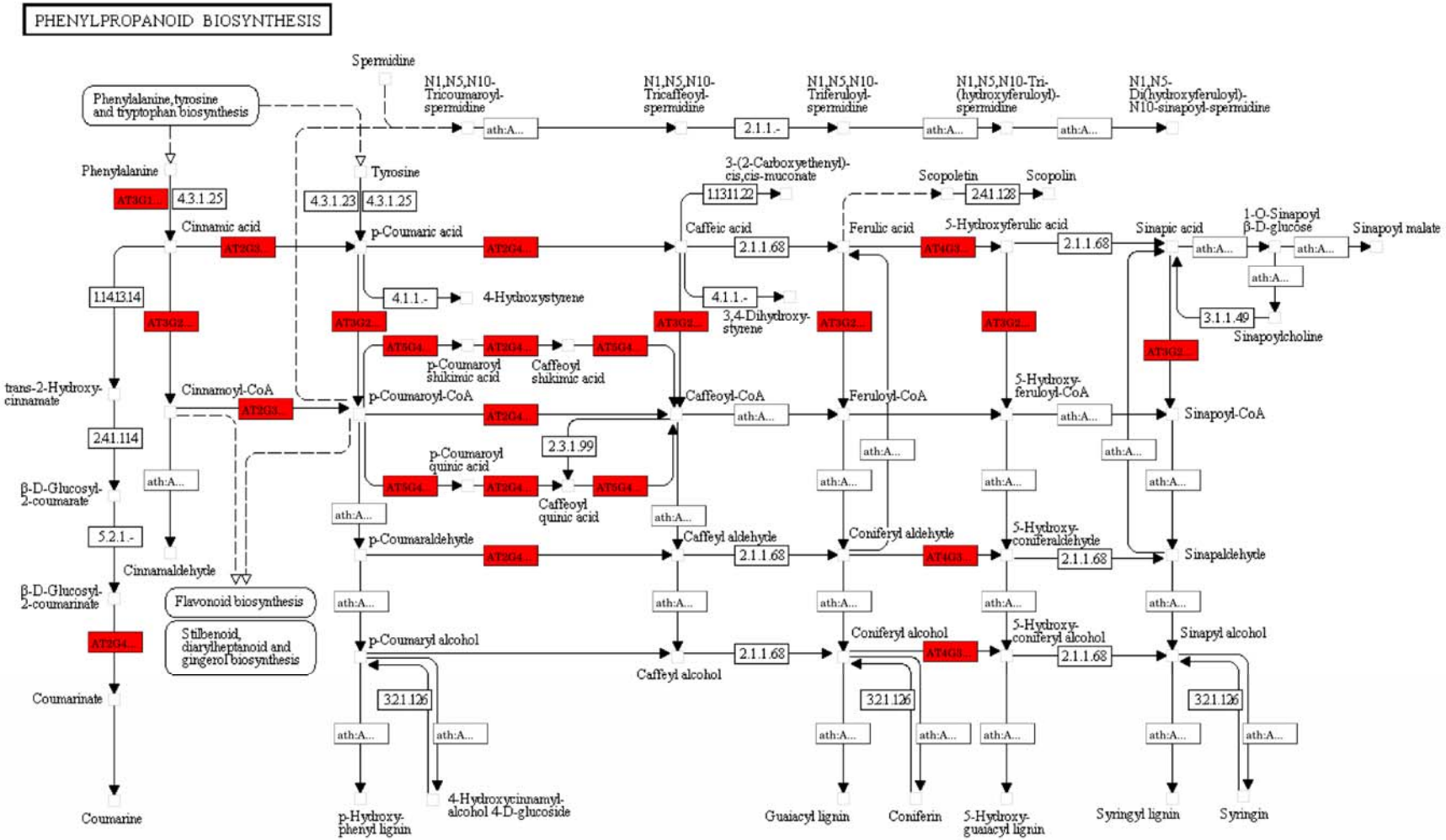


**Figure 36. The figure displays the phenylpropanoid pathway. Enzymes whose expressions is regulated by ProSys are indicated in red providing their correspondent Arabidopsis TAIR identifier (table 5).**

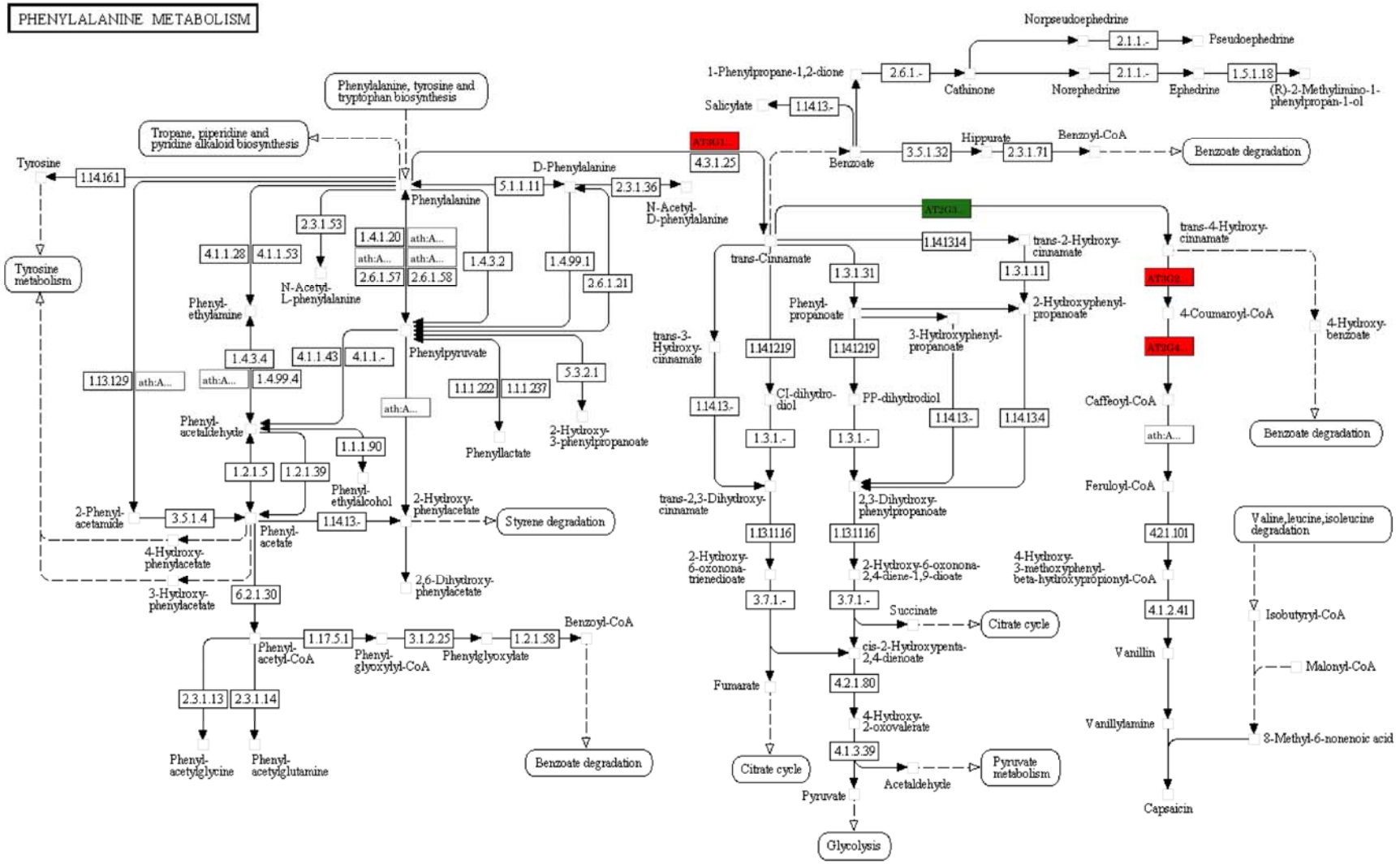


**Figure 37. The figure displays the phenylalanine metabolism in which ProSys-regulated enzymes are coloured in red if up-regulated and in green if down-regulated.**

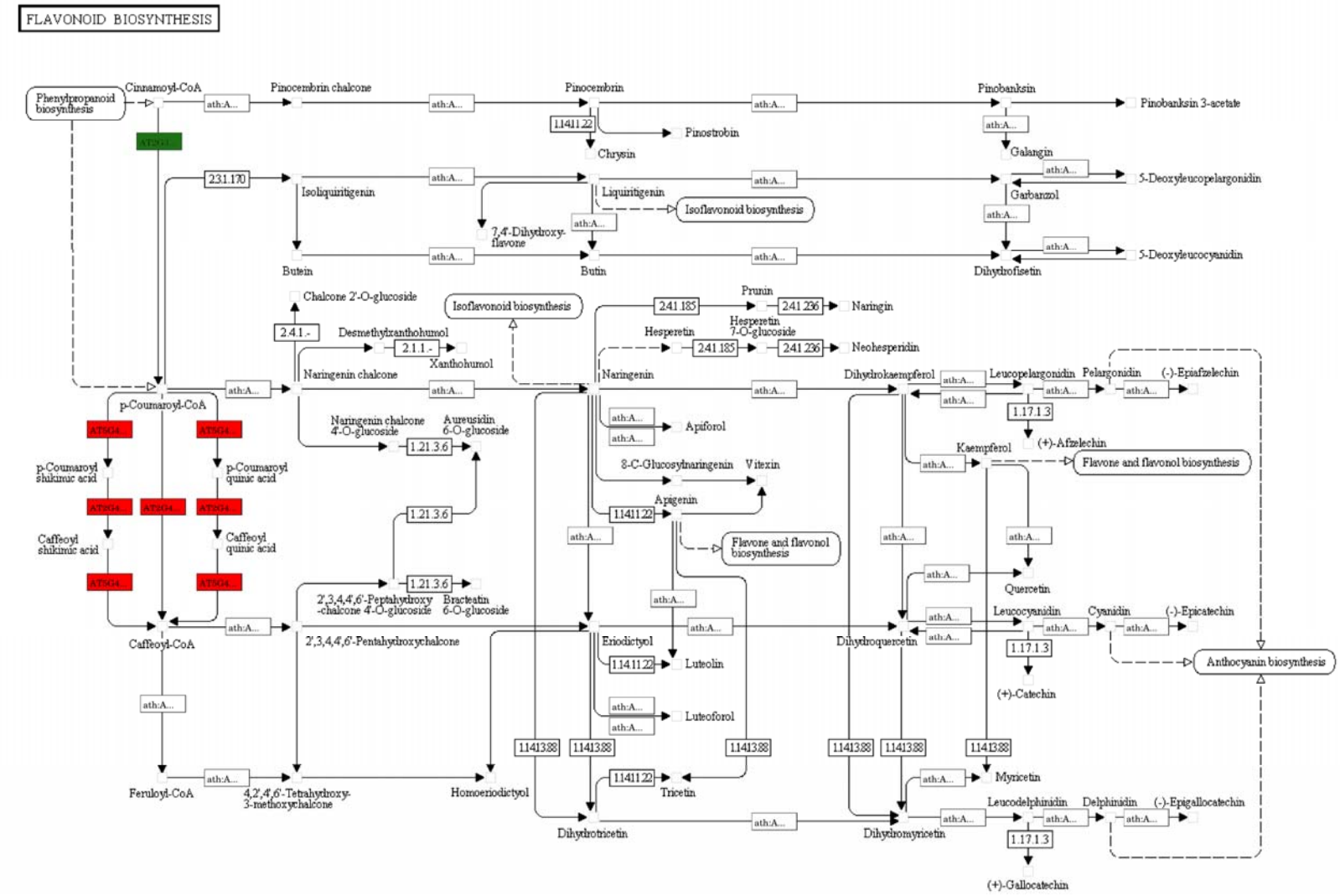


**Figure 38. The figure displays the flavonoid biosynthesis. ProSys-regulated enzymes are coloured in red if up-regulated and in green if down-regulated.**

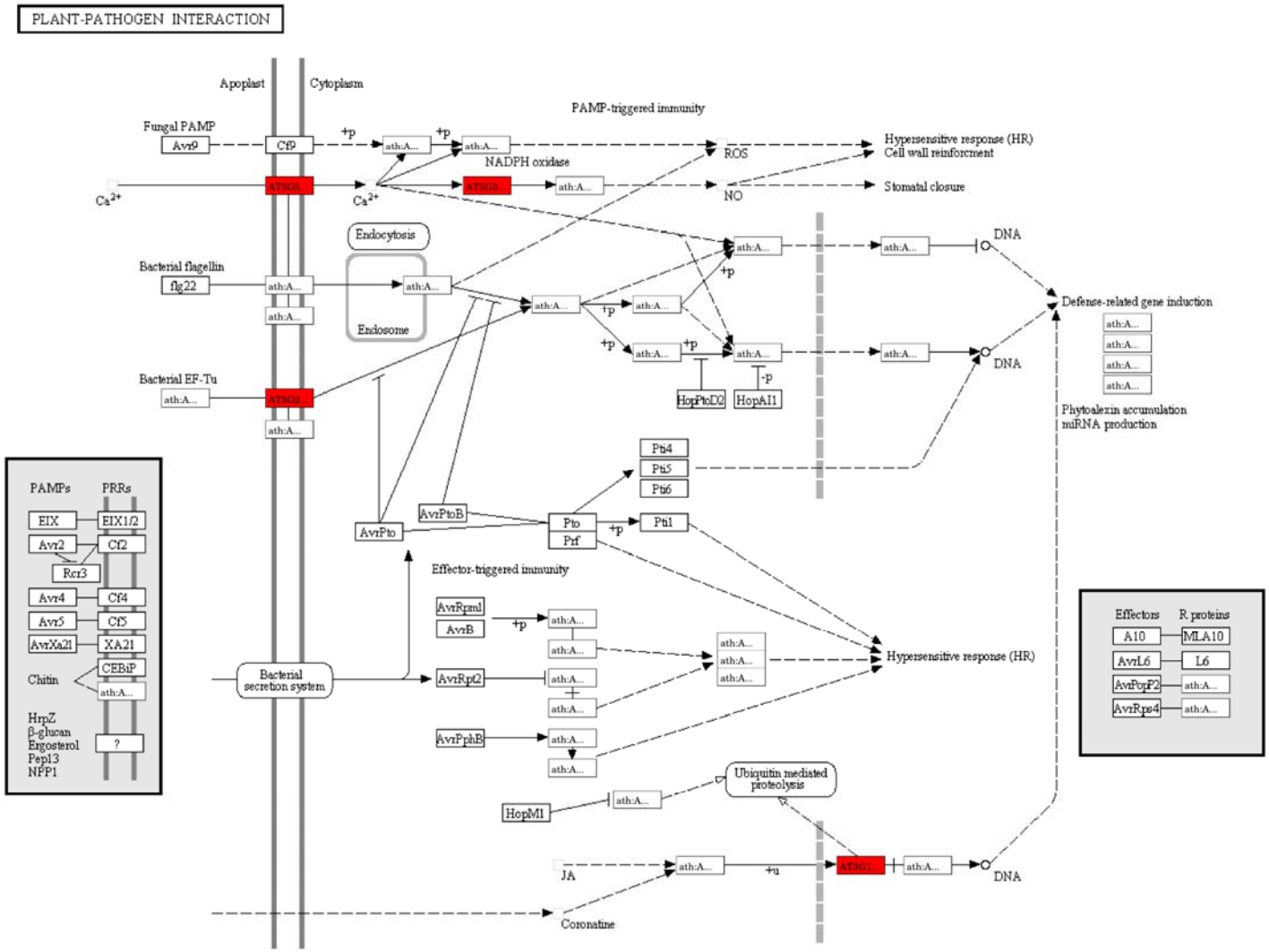


**Figure 39. The figure displays proteins involved in plant-pathogen interaction pathway designed with Painomics software (www.paintomics.org).**

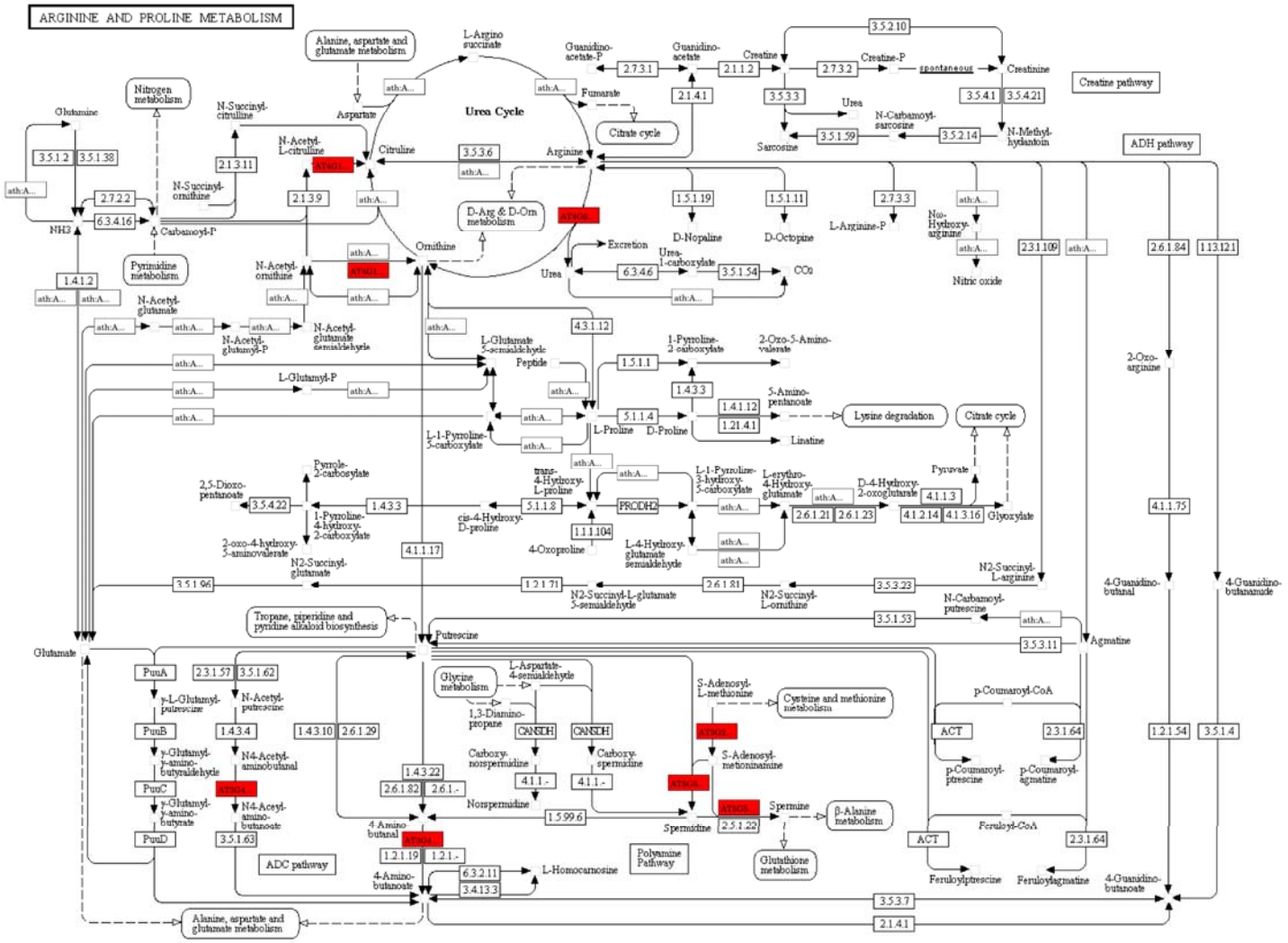


**Figure 40. The image displays the arginine and proline metabolism in which enzymes correspondent to ProSys-regulated genes are underlined.**

The accumulation of ornithine is promoted (fig. 40) since the strong up-regulation of Peptidase M20/M25/M40 family protein (AT4G17830) and arginase (AT4G08900). Ornithine is a central compound in this pathway and it is converted in putrescine that is involved in reactions catalyzed by aldeyde dehydrogenase (AT3G48000), S-adenosylmethionine decarboxylase (AT3G25570) and spermidine synthase 3 (AT5G53120) leading to spermine and spermidine biosynthesis. Putresine, spermine and spermidine are part of polyamine (PAs) group. PAs are precursors for secondary metabolites and conjugated with phenolic acids which have been linked with plant–pathogen defence responses (Walters *et al.,* 2003). PAs are also known to enhance the tolerance to environmental stresses such as salinity, chilling, drought, potassium deficiency (Wimalasekera *et al.,* 2011). So ProSys-regulated genes coding for proteins that are very relevant in these pathways associated to abiotic and biotic stresses have been identified. Moreover, they seem to be the main players of these responses.

### 3.9 *In silico* Network of protein-protein interactions in RSYS plants

Since prosystemin is a molecule involved in signal transduction, the study of the interactions between ProSys-regulated proteins is interesting to clarify the dynamics of the defence responses. To this aim, the list of 309 Arabidopsis proteins (table A2), corresponding to the RSYS differentially expressed sequences, was submitted to the Arabidopsis Interactions Viewer, a on line tool available at the University of Toronto website (http://bar.utoronto.ca/). The Arabidopsis Interactions Viewer queries a database of 70944 predicted and 28291 confirmed Arabidopsis interacting proteins. The list of interest can be easily submitted in an opposite box of the tool getting back a network of proteins constituted by all proteins of the Arabidopsis interactome that interact with the proteins contained in the list submitted. In the output network from BAR, nodes (proteins) are coloured based on the sub-cellular compartment, their size is correlated to their connection degree (see mat and met section, par. 2.7.1) while edges (interactions) thickness is related to the importance of the interaction. The attribute file, which describes all nodes in the network, was built matching all Arabidopsis protein annotations downloaded from TAIR website (www.arabidopsis.org) with all nodes in the network produced with the BAR tool.

The network and the attribute files were loaded in Cytoscape 2.8.1 (www.cytoscape.org) and checked in its RSYS protein list content. This network was found to be low representative since only 95 proteins of the 309 RSYS list were found. To attach more nodes on the previous network the interactome downloaded from TAIR website (www.arabidopsis.org) was used. In fact this approach showed a better match with RSYS protein list than the interactome from BAR. In this way 100 more nodes were added obtaining a well representative protein network including 2066 nodes, 2661 edges and 195 proteins of RSYS list. Nodes were coloured based on the sub-cellular compartment and whose of RSYS list were underlined with a triangle shape and a yellow colour (fig. 41).

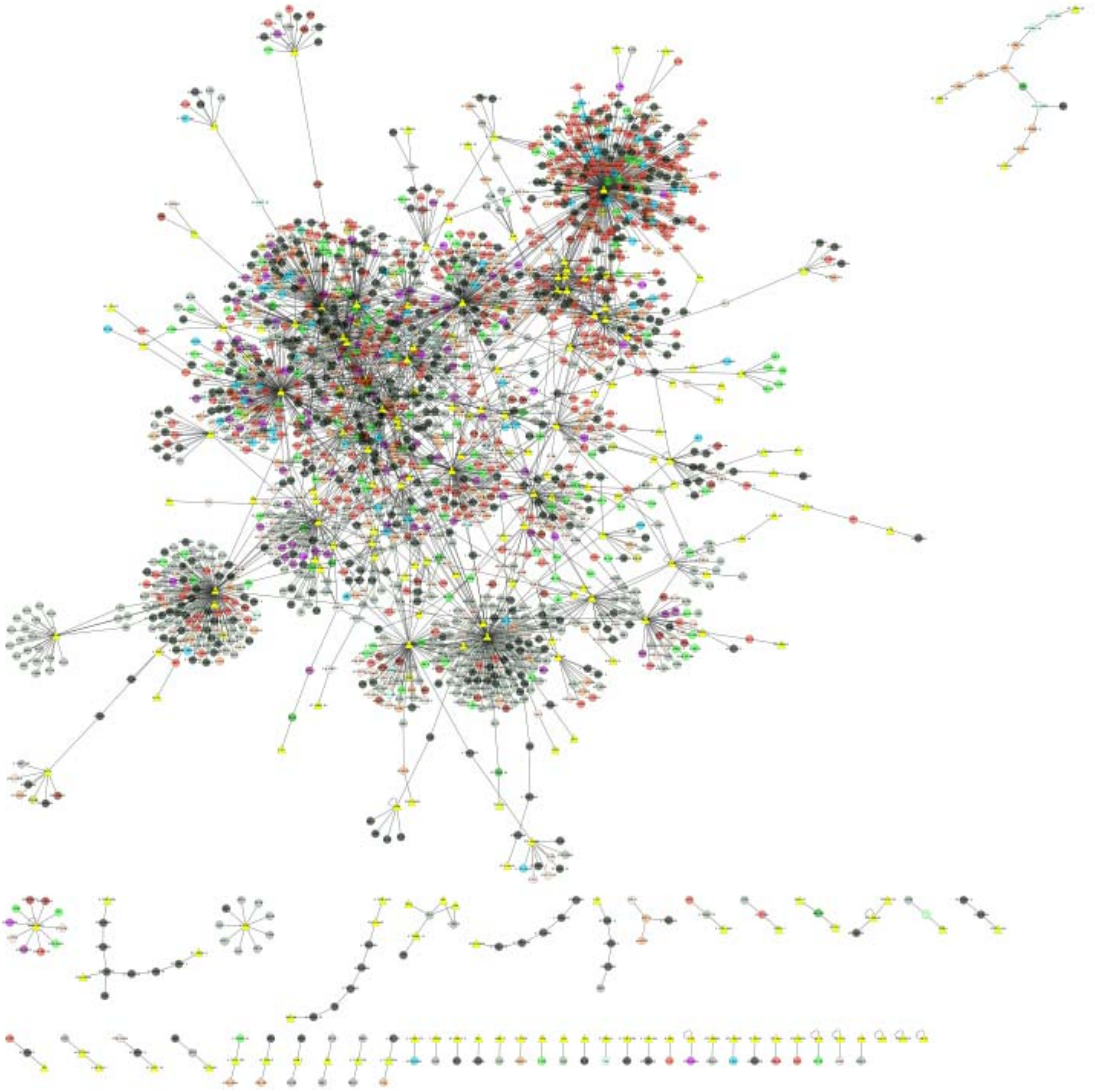

**Figure 41. The image displays the RSYS network obtained with Cytoscape 2.8.1 software. Nodes are coloured based on the sub-cellular compartment. In yellow and with a triangle shape nodes corresponding to ProSys-regulated genes are indicated.**

As shown in fig. 41, proteins coded by the differentially expressed sequences in RSYS samples are located in the centre of the main relevant nodes of the network indicating that ProSys over-expression affects proteins involved in the most important interactions between the proteins of the network.

### *3.9.1 Network topology and parameters analysis*

The network topology can be analyzed with the graph theory which refers to some parameters in describing networks. The global network parameters were firstly analyzed in Cytoscape using the Network Analyzer tool (fig. 38).

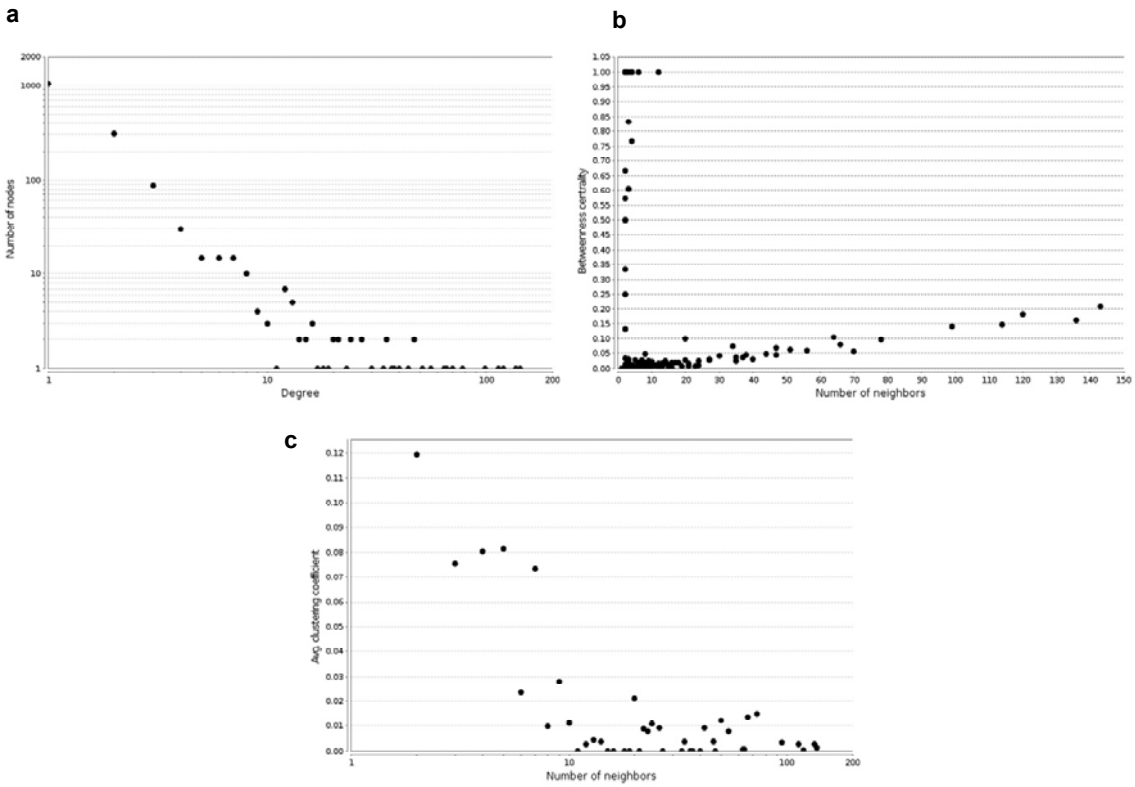


**Figure 42. The image shows the network parameters analysis carried out in Cytoscape. a) Connection degree distribution; b) Betweenness centrality; c) Average Clustering Coefficient distribution.**

Parameters investigated are: the connection degree distribution refers to the distribution of the average of the connection degrees in the network; the betweenness centrality indicates the centrality of a node in the network and describes the betweenness distribution of all nodes with *k* neighbors; the average of clustering coefficient distribution describes the distribution of clustering

coefficient of all nodes with *k* neighbors. For more information about network parameters see mat and met section, par 2.7.1.The node degree distribution (fig. 42a) reveals a “scale-free” network; this means that there are many nodes with a low degree, so with few interactions within the network, and few nodes with a high degree, so highly connected. This means that there are some central nodes that are very relevant for maintain network structure. This observation is supported by the beetweenness centrality that is a specification of the betweenness concept. It refers to the amount of control that a node can exert on the interactions of other nodes in the network and it is defined by a ratio that can assume values between 0 and 1. So, a node with betweenness centrality of 1 is very important in the network and it is a good candidate to be a “hub”. An “hub” is a central node and if it is removed the network could change its shape, in fact removing more than one hub could destroy the network. Fig. 42b shows the distribution of beetweenness centrality referred to the number of neighbours. The comparison of nodes with the same number of neighbours in RSYS network evidenced a few nodes with betweenness centrality of 1, several nodes of an intermediate value and many of 0 value. Also the average of clustering coefficient distribution (fig. 42c) was consistent with these two previous parameters. In fact, the comparison of nodes with the same number of neighbours, resulted in the identification of some with an high attitude to be part of a cluster, showing a high average of clustering coefficient, and many other for which this value is very low. All toghether these parameters indicated that the RSYS network is a scale-free network (Minguez *et al.,* 2009) with some nodes that can be defined as “hub” with central position and role in the shape of the network. Several criteria have been used to define hubs in protein network, all generally referring to the node degree. Referring to Aragues and collaborators (2007) nodes with degree greater than 20 were labelled as hubs and are indicated in table 11.

**Table 11. Hubs identified in RSYS network.**

| TAIR identifier | Gene Symbol | Name | Compartment | Description | Node Degree |
|---|---|---|---|---|---|
| AT1G14700 | PAP3 | ATPAP3_PAP3__purple acid phosphatase 3 | vacuole | protein serine/threonine phosphatase activity | 275 |
| AT3G22840 | ELIP | ELIP_ELIP1__Chlorophyll A-B binding family protein | chloroplast | Chlorophyll A-B binding family protein | 269 |
| AT3G26590 | AT3G26590 | MATE efflux family protein | vacuole | drug transmembrane transporter | 157 |
| AT4G35800 | NRPB1 | NRPB1_POL_II_LS_RNA _LSRNA_POL_II_LS_RPB1__RNA polymerase II large subunit | nucleus chloroplast | regulation of transcription | 146 |
| AT5G37780 | CAM1 | ACAM-1_CAM1_TCH1__calmodulin 1 | nucleus | calmodulin 1 | 131 |
| AT1G10070 | ATBCAT-2 | ATBCAT-2_BCAT-2__branched-chain amino acid transaminase 2 | chloroplast | metabolic process | 127 |
| AT1G10090 | AT1G10090 | Early-responsive to dehydration stress protein (ERD4) | membrane | Early-responsive to dehydration stress protein (ERD4) | 127 |
| AT4G36490 | ATSFH12 | ATSFH12_SFH12__SEC14-like 12 | unknown | actin cytoskeleton organization | 84 |
| AT5G26250 | AT5G26250 | Major facilitator superfamily protein | membrane | carbohydrate transmembrane transporter activity | 77 |
| AT3G20390 | AT3G20390 | endoribonuclease L-PSP family protein | chloroplast mitochondrion | endoribonuclease L-PSP family protein | 73 |
| AT3G10330 | AT3G10330 | Cyclin-like family protein | nucleus | regulation of cell cycle | 65 |
| AT2G41380 | AT2G41380 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein | mitochondrion | methyltransferase activity Ontology: molecular function | 60 |
| AT3G01160 | AT3G01160 | Unknown | unknown | unknown | 58 |
| AT1G05830 | ATX2 | ATX2_SDG30__trithorax-like protein 2 | nucleus | regulation of transcription | 51 |
| AT4G30660 | AT4G30660 | Low temperature and salt responsive protein family | unknown | Low temperature and salt responsive protein family | 48 |
| AT1G66410 | CAM4 | ACAM-4_CAM4__calmodulin 4 | membrane | Calmodulin 4 | 47 |
| AT5G53120 | ATSPDS3 | ATSPDS3_SPDS3_SPMS__spermidine synthase 3 | unknown | spermidine synthase 3 | 44 |
| AT3G10050 | OMR1 | OMR1__L-O-methylthreonine resistant 1 | chloroplast | isoleucine biosynthetic process | 39 |
| AT1G12900 | GAPA-2 | GAPA-2__glyceraldehyde 3-phosphate dehydrogenase A subunit 2 | chloroplast | metabolic process | 37 |
| AT5G15240 | AT5G15240 | Transmembrane amino acid transporter family protein | membrane | transmembrane transport | 37 |
| AT1G07430 | HAI2 | HAI2__highly ABA-induced PP2C gene 2 | nucleus | serine threonine kinase activity | 35 |
| AT3G12490 | ATCYS6 | ATCYS6_ATCYSB_CYSB__cystatin B | unknown | cysteine biosynthesis | 35 |
| AT3G28030 | UVH3 | UVH3_UVR1__5'-3' exonuclease family protein | nucleus | DNA repair protein UVH3 | 31 |
| AT2G26695 | AT2G26695 | Ran BP2/NZF zinc finger-like superfamily protein | nucleus | DNA binding | 31 |
| AT4G25150 | AT4G25150 | HAD superfamily; subfamily IIIB acid phosphatase | unknown | unknown | 28 |
| AT5G53390 | AT5G53390 | O-acyltransferase (WSD1-like) family protein | unknown | unknown | 26 |
| AT2G45660 | AGL20 | AGL20_ATSOC1_SOC1__AGAMOUS-like 20 | nucleus | maintenance of inflorescence meristem identity | 25 |
| AT1G17840 | WBC11 | ABCG11_ATWBC11_COF1_DSO_WBC11__white-brown complex homolog protein 11 | membrane | protein folding | 24 |
| AT5G53130 | ATCNGC1 | ATCNGC1_CNGC1__cyclic nucleotide gated channel 1 | membrane | cation channel activity | 23 |
| AT2G30490 | ATC4H | ATC4H_C4H_CYP73A5_REF3__cinnamate-4-hydroxylase | membrane | phenylpropanoid biosynthesis | 22 |
| AT3G55480 | PAT2 | PAT2__protein affected trafﬁcking 2 | endomembrane system | transport activity | 21 |
| AT4G16520 | ATG8F | ATG8F__Ubiquitin-like superfamily protein | vacuole | Ubiquitin-like superfamily protein | 20 |
| AT2G40890 | CYP98A3 | CYP98A3__cytochrome P450; family 98; subfamily A; polypeptide 3 | mitochondion | cytochrome P450 | 20 |

In order to evaluate the statistical significance of these parameters, an enrichment analysis was carried out with SNOW, Studying Networks in the Omic World, an online free software included in Babelomics package (www.babelomics.org). This software compares the distributions of node, edge and graph parameters of the protein list under investigation against the distribution of the same parameters of thousand random lists with the same size. These random lists are produced by the software using the reference TAIR interactome provided. So, SNOW performs a statistical evaluation of the network parameters by comparison with a random network using the Kolmogorov-Smirnov test.

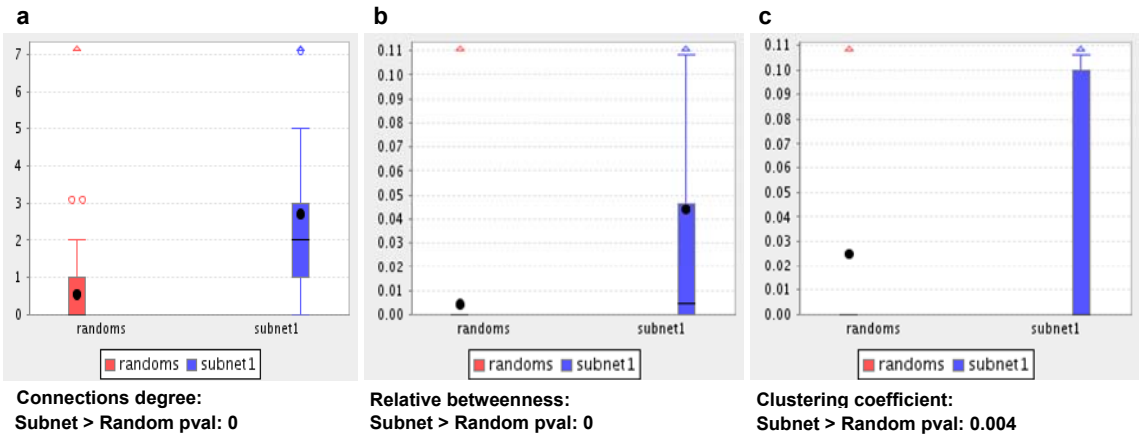


**Figure 43. The image shows the comparison between RSYS network and the random network parameters by SNOW analysis. a) Connections degree; b) Betweenness; c) Clustering coefficient.**

For all parameters under analysis, the Kolmogorov-Smirnov test (Minguez *et al.,* 2009) found a statistical significance comparing the RSYS network and the random network obtained from the reference interactome provided (fig. 43). Besides, respect to random networks, RSYS network resulted more connected (connection degree, fig. 43a), with a higher number of central nodes (relative betweenness. fig. 43b) with higher attitude to form clusters (clustering coefficient, fig. 43c). These analysis demonstrate that the topology of RSYS network is not casual or random, but has a biological relevance. In order to study the biological function of the most important nodes a collapsing strategy was used to simplify the RSYS network (fig. 44).

**Figure 44. The image shows the RSYS collapsed network obtained by Cytoscape plus MetaNode plugin.**

The MetaNode plugin (http://chianti.ucsd.edu/cyto_web/plugins/) was imported in Cytoscape 2.8.1 to collapse proteins with the same functions and the same sub-cellular localization in a unique metanode. To distinguish metanodes from the other nodes in the network, a rectangle shape was attributed to the previous ones. The collapsing strategy provided a very little simplification of RSYS network since only nodes sharing the same function and the same compartment were collapsed. Often, nodes sharing the same function were located in different compartment so they have not been fused in a metanode.

Zooming on some hubs (table 11) of RSYS network, several interesting interactions between proteins involved in responses against different stress agents

were found. The following proposed clusters are an example of the centrality of ProSys-regulated proteins in the crosstalk between different defence pathways.

### *3.9.2 The ERD4 node*

ERD4 (*Early Responsive to Dehydration*) protein (fig. 45) is a membrane protein involved in stress responses. *ERD4* sequence analysis indicated that its gene product may function in drought tolerance and response in *Arabidopsis* (Kyiosue *et al.,* 1994). Sequencing cDNA coding for ERD proteins revealed that three ERDs were identical to those of HSP cognates (Athsp70-1, Athsp81-2, and ubiquitin extension protein), three others are associated to gluthadione-S-transferase (GST), ERD5 is a proline dehydrogenase while ERD6 is a sugar transporter and many other proteins of this family have been not yet identified (Taji *et al.,* 1999). ERD4 is induced by ProSys and is involved in several interesting interactions in RSYS network (fig. 45). It shows interactions with other elements involved in abiotic stress responses, such as another early responsive to dehydration protein ERD1 (AT2G03250) that is a ClpA/B ATP-dependent protease, a low temperature and salt responsive protein (AT1G57550) and a heat shock cognate protein 1 (AT-HSC70-1). Located in the cytoplasm there is a DNA-J chaperonine (AT5G49060) that is involved in protein folding and signal transduction in response to environmental stresses. Boddu and collaborators (2006) reported the induction of a DnaJ-related chaperone protein in barley 72 h after *Fusarium* inoculation, but it is not clear what role DnaJ-like proteins play in the interaction of *Fusarium* with its cereal hosts. Recently, Alfenas-Zerbini and collaborators (2009) found its induction in tomato after potyvirus infection confirming the DNA-J involvement in abiotic and biotic responses. ERD4 is also connected to annexin 1 (ANNAT1) that is one of eight proteins of the homonymous family in *A. thaliana*. Annexins are calcium-dependent phospholipid-binding proteins with a peroxidase activity, so they play an important role in maintaining calcium homeostasis in the cell and calcium signalling plus an involvement in the oxidative stress (Górecka *et al.,* 2007). ERD4 shows interactions with several players of signal transduction, such as kinases and NADPH dehydrogenase that have been collapsed in metanodes. More signalling-related proteins are located on the membrane, such as SRF7 that is a member of STRUBBELIG-RECEPTOR FAMILY (SRF), a class of putative leucine-

rich repeat receptor-like kinases (Eyüboglu *et al.,* 2007). In this class of receptors ATBRI1 is also included. Phospholipase D gamma 3 (PLDGAMMA3) is responsible of fatty acids release from membranes and is also associated to this biological process. Besides, kinases involved in signal transduction are also located in other sub-cellular compartments, such as several nuclear CBL-interacting kinase (CIPK) and the cytosolic yeast YAK1-related gene (YAK1).

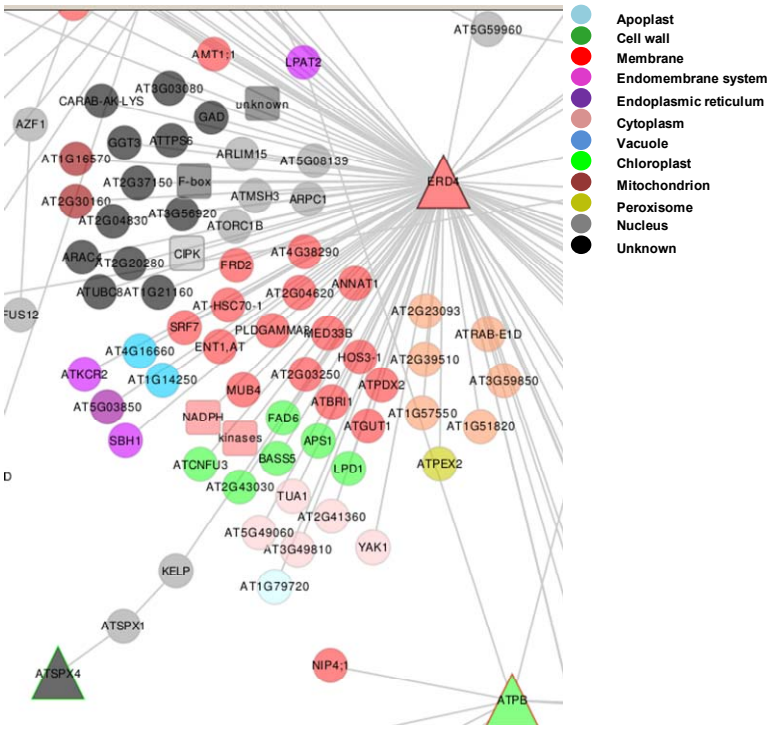


**Figure 45. The image displays the ERD4 node and its neighbourhood in which colours indicate the sub-cellular compartment (see legend in the picture), nodes with rectangle shape are metanodes, nodes with triangle shape are in RSYS list and their border colour indicate the type of regulation (red if up-regulated, green if down-regulated, black if the colour attributed based on the regulation is the same colour of the node based on the compartment).**

Among nuclear proteins, there are interactions with proteins related to DNA repair such as ATORC1, ARPC1, ARLIM15 and ATMSH3. An interesting interaction is with FPS (AT1G51820), *farnesyl pyrophosphate synthase,* an enzyme involved in terpenoids biosynthesis demonstrating a direct connection between abiotic and biotic indirect responses. For more information about RSYS proteins in this node see table A2.

### *3.9.3. Connections between CYP98A3, ATC4H and HAI2*

The interactions between cinnamate-4-hydroxylase (ATC4H) and the cytochrome P450 98A3 (CYP98A3) that are enzymes involved in the phenylpropanoid pathway, previously described in par. 3.4.4, with a protein phosphatase 2C (HAI2) have been found in RSYS network (fig. 46). Seventy-six *Arabidopsis* genes were identified as PP2C-type phosphatase candidates and associated to ABA- and MAPK-signalling, but functions have been assigned to only a few PP2C genes. (Schweighofer *et al.,* 2004). In fact, as shown in fig. 46 the protein phosphatase 2C (HAI2) interacts with many signalling molecules such as CBL-interacting kinases (CIPK), AGC kinase 1.7 (AGC1.7), endoribonuclease/protein kinase IRE1-like (ATIRE1-2), histidine kinase (AHK1) and casein kinase II beta subunit (CKB4). This picture is a portrait of some interconnected defence pathways since the linkage between proteins active against salt and osmotic stresses, responses against pathogens and herbivory are directly connected. CYP98A3 and ATC4H share several membrane interactors mainly associated to transport, like ATPROT1 for proline, ATPTR1 for peptides, ATNTR1 for nitrate and ATPUP1 that is a purine permease. Aquaporin 2 (PIP2;8) is also a common interactor between these two characters of phenylpropanoid pathway. Aquaporins (AQPs) were discovered as channels facilitating water movement across cellular membranes. Some AQPs can conduct a wide range solutes, such as urea or glycerol, ROS such as carbon dioxide, nitric oxide and hydrogen peroxide and the metals like arsenic, boron and silicon (Hatchez *et al.,* 2010) and they have been associated to $Ca^{2+}$ homeostasis (Gilliham *et al.,* 2011). MLO-like protein 4 (ATMLO4) is a member of seven-transmembrane domain MILDEW RESISTANCE LOCUS O family in *Arabidopsis*. The barley MLO protein is thought to modulate defense responses to the biotrophic *Blumeria graminis f. sp. hordei (Bgh)* via a vesicle-associated and SNARE protein–dependent mechanism (Panstruga *et al.,* 2005). Homozygous mutant (*mlo*) alleles of the *Mlo* gene confer broad spectrum disease resistance to the biotrophic *Bgh* (Jørgensen, 1992). Interestingly, AtMLO proteins confer resistance to the necrotrophic *Alternaria spp.* and the hemibiotrophic *Phytophtora infestans* promoting apoptosis (Consonni *et al.,* 2006).

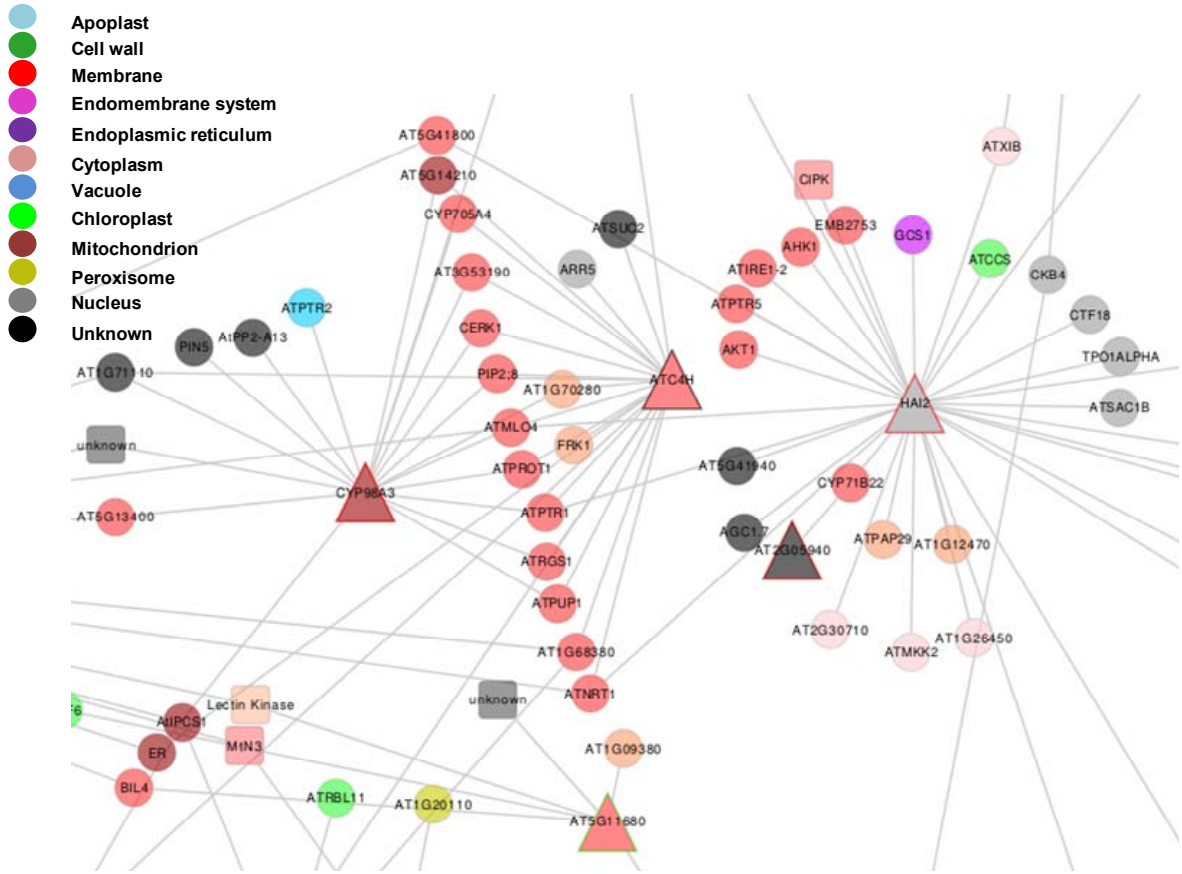


**Figure 46. The image displays CYP98A3, ATC4H and HAI2 nodes in which colours indicate the sub-cellular compartment (see legend in the picture), nodes with rectangle shape are metanodes, nodes with triangle shape are in RSYS list and their border colour indicate the type of regulation (red if up-regulated, green if down-regulated, black if the colour attributed based on the regulation is the same colour of the node based on the compartment).**

Another interesting interaction of the ProSys-induced CYP98A3 is with the phloem protein AtPP2. This is a phloem lectin (Dinant *et al.,* 2003) whose over-expression confers *Arabidopsis* resistance to the green peach aphid *Myzus persicae* (Zhang *et al.,* 2011). The chloroplastic CCS copper chaperone for superoxide dismutase 1 (ATCCS) is involved in protein folding during oxidative burst. Its involvement in signal transduction is explicated inside RSYS network through the interaction with HAI2 and its-related kinases For more information about RSYS proteins in this node see table A2.

### *3.9.4 Calmodulin 1 node*

Calmodulin 1 (CAM1) is coded by a ProSys-induced gene and its activity obviously concerns the signal transduction and the calcium-mediated cascade. In fig. 47, the connection with a large amount of signalling molecules is observed. First of all, CAM1 interacts with several other calmodulins collapsed in a metanode. Besides, there are several signalling-related metanodes, such as leucine-rich repeat receptor-like kinases (LRR-RLKs), MAP kinases (MAPK), ser/arg-rich protein kinases (RPKs) and integrin kinases. Other kinases in CAM1 neighbourhood are receptor-like protein kinase-related family protein (CRK9 and CRK21), CBL-interacting protein kinase 6 (ATCIPK6), protein kinase 2B (APK2B) and cyclin-dependent protein kinase 6 (CDPK6).

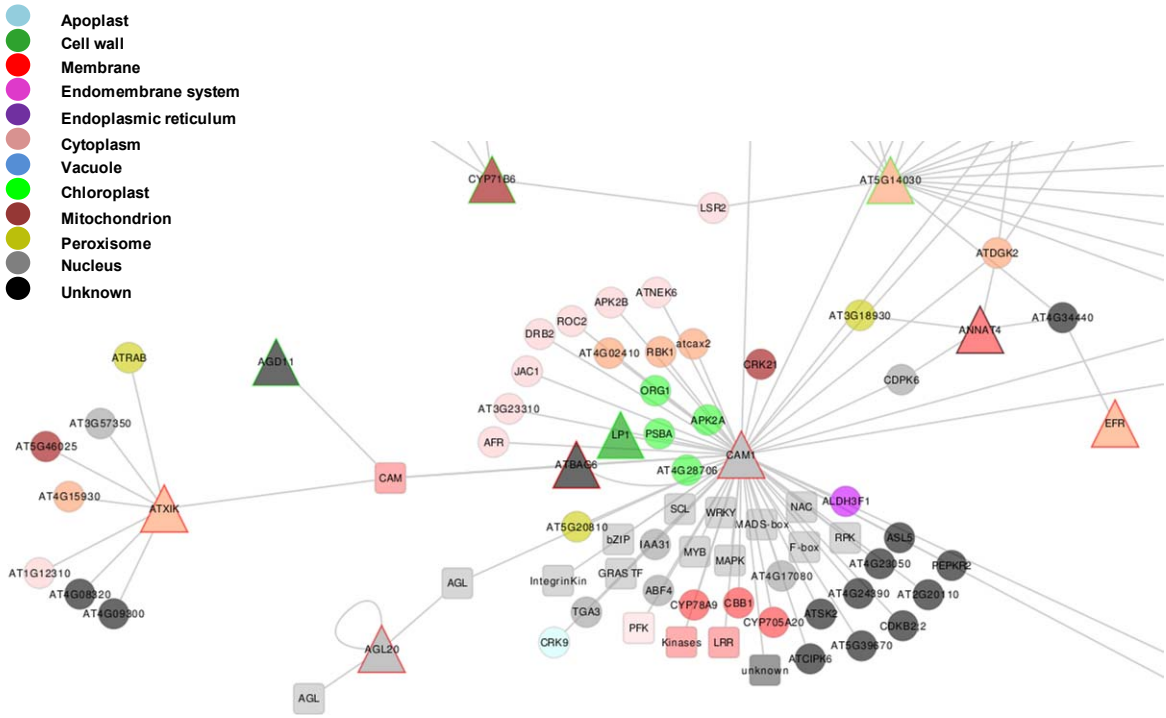


**Figure 47. The image displays the CAM1 node and its neighbourhood in which colours indicate the sub-cellular compartment (see legend in the picture), nodes with rectangle shape are metanodes, nodes with triangle shape are in RSYS list and their border colour indicate the type of regulation (red if up-regulated, green if down-regulated, black if the colour attributed based on the regulation is the same colour of the node based on the compartment).**

CAM1 is connected with many transcriptional factors involved in the activation of defence genes, such as AGAMOUS-like (AGL), bZIP, MYB and WRKY families (Deslandes and Rivas, 2011). TGA transcription factors are implicated as

regulators of pathogenesis-related (PR) genes because of their physical interaction with the known positive regulator, nonexpresser of PR gene1 (*NPR1*; Kesarwani *et al.,* 2007). NAC domain transcription factors are positive regulators of ABA signalling inducing the expression of ERD11 (early responsive to dehydration, par. 3.7.2), cold-responsive 47 (COR47) and RD29b (responsive-to-desiccation 29b; Jensen *et al.,* 2010). Still linked to ABA signalling, there is the interaction with ABF4, ABRE binding factor 4 where ABRE is a promoter which interacts with DREB2A transcription factor responsible of the activation of drought-responsive genes (Kim *et al*., 2011). CAM1 relationship with auxin signalling, recently associated to resistance against *B. cinerea* (Llorente *et al.,* 2008), is mediated by indolacetic acid 31 (IAA31) and SAUR-like auxin-responsive protein family (AT5G20810). Connections with phenylpropanoid and brassinosteroid pathway are also observed via the the shikimate kinase 2 (ATSK2) and the cell elongation protein CBB1, respectively. A linkage with plant responses against viruses is also found in this node looking at the interactions with a double-strand RNA binding protein (DRB2) and an argonaute family protein (AGO1). These are members of RNA interference machinery active in two phases of target mRNA degradation: DRB2 is involved in the recognition of dsRNA while AGO1 is part of an enzymatic complex which cleaves target mRNAs (Hannon, 2002). As shown in fig. 47, calmodulins interact with cytoskeleton components, such as myosin (ATXIIK) that is another protein coded by a ProSys-induced gene. The interaction between plant cells and pathogens triggers a range of highly dynamic plant cellular responses including reorganization of the cytoskeleton (Yao *et al.,* 2011). Microfilaments and microtubules are necessary for plants to block fungal penetration (Genre and Bonfante, 2002). The interaction between CAM1 and ATXIIK has an intermediary, the BCL-associated anathogene 6 (ATBAG6) that has been associated to MAP signalling cascade (Ueda *et al.,* 2004). For more information about RSYS proteins in this node see table A2.

### *3.9.5 PAP3 node*

The last example of defence-related nodes in RSYS network is the purple acid phosphatase (PAP3) one, an up-regulated serine/threonine kinase located on vacuolar tonoplast which represents a key molecule in signal transduction (fig. 48).

Acid phosphatases are ubiquitous and abundant in plants, animals, fungi, and bacteria and are believed to function in the production, transport and recycling of phosphorus ($P_i$) (Bozzo *et al.,* 2002). They are induced under various stresses, such as water deficiency, salinity stress, and nutritional $P_i$-deficiency (Duff *et al.,* 1994). PAPs represent a distinct class of nonspecific acid phosphatase containing binuclear transition metal centres (Vincent *et al.,* 1992). It has been shown that PAPs may also display peroxidase activity generating ROS and the consequent hypersensitive reaction (Del Pozo *et al.,* 1999).

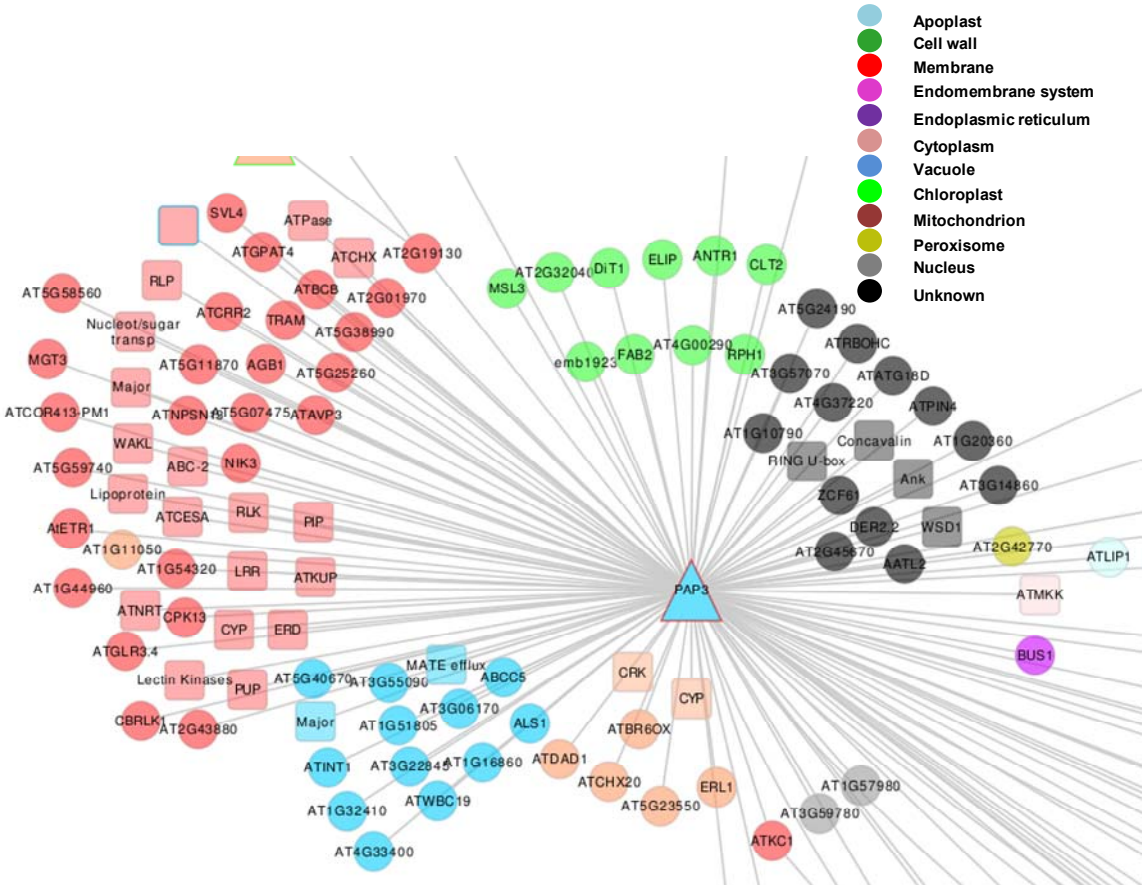


**Figure 48. The image displays the PAP3 node and its neighbourhood in which colours indicate the sub-cellular compartment (see legend in the picture), nodes with rectangle shape are metanodes, nodes with triangle shape are in RSYS list and their border colour indicate the type of regulation (red if up-regulated, green if down-regulated, black if the colour attributed based on the regulation is the same colour of the node based on the compartment).**

PAP3 central role in signal transduction is clear looking at the interaction with more than 30 kinases divided in MAPK, CBL-interacting, lectin and receptor-like kinases, but also several LRR-RLK domain proteins, receptor-like proteins (RLP), histidine kinase (ATETR1) and so on. An interesting kinase class include the cell

wall associated kinase (WAK) and the WAK-like kinase (WAKL). This represent a unique RLK subfamily whose 26 members are excellent candidates for signaling molecules that directly link and communicate between the cell wall and the cytoplasm (Hou *et al.,* 2005). Expression of WAK1 can be induced by pathogens and the induced expression is required for *Arabidopsis* to survive during pathogenesis (He *et al.*, 1998). Besides, WAK1 is an aluminum early responsive gene and its over-expression results in aluminum tolerance (Sivaguru *et al.*, 2003). Another RLK subfamily is composed by 14 proteins referred as NIK receptors. The Arabidopsis NSP-interacting kinase 1, NIK1 (AT5G16000), NIK2 (AT3G25560), and NIK3 (AT1G60800) are virulence targets of the bipartite geminivirus nuclear shuttle protein, NSP (Fontes *et al.,* 2004). NSP from the cabbage leaf curl virus interacts with all three NIKs from Arabidopsis to suppress their kinase activity (Fontes *et al.,* 2004). The acyl-desaturase 7 (FAB2) also represents an important character of defence responses against viruses, bacteria and insects since it promotes the crosstalk between SA- and JA-mediated pathways. Focusing on pathogen elicited responses, ATDAD1 regulates apoptosis while RPH1 and CLT2 are associated to resistance against *Phytophtora infestans* and *oomycetes*, respectively. Other interesting defence molecules located in PAP3 neighbourhood are the hydroxyproline-rich systemin glycopeptides which cooperates with systemin in the herbivory-induced responses (Narvaez-Vasquez *et al*., 2007), ankyrin proteins, lipases, several sugar and ions transporters that have been recently associated to aphid responses due to the mobilization of nutrients (Moran *et al.,* 2005), oxidases involved in the oxidative burst and transcription factors involved in the brassinosteroid-mediated pathway. For more information about RSYS proteins in this node see table A2.

# 4. DISCUSSIONS AND CONCLUSIONS

Reduction of pesticide distribution is one of the major objectives in sustainable agriculture, and is largely being addressed by adopting environmentally-safe products. Moreover, accordingly to (Oerke *et al.,* 2004) the predicted increment of the world population up to 10 million during the next four decades, requires a remarkable increase of food production in an environmentally sustainable way. To this aim, the scientific community is focused on the reduction of losses acting on their origins. Oerke and collaborators (2004) attributed the 29% of losses of the main cash crops to insects and pathogens. During 1990s thanks to the spreading of DNA recombinant technology, engineering crop plants for endogenous resistance to pests has been one of most used strategy. During those years, genetically modified maize, potato and cotton plants expressing genes encoding the entomocidal δ-endotoxin from *Bacillus thuringiensis* (Bt) were the main examples of transgenic crops enhanced in resistance against pests. Since then several combinations of Bt toxins were used in order to overcome possible resistances developed by insects. Although plants expressing Bt toxins have been successful in protecting agricultural crops against insect pests, there is still a need to develop further strategies for engineering insect resistance (Ferry *et al.,* 2004). Biotech crops could answer this necessity, but they have to interface with the negative public opinion (Jones *et al.,* 2011). Transgenes under the control of regulated promoters or the use of recombinase *Cre/Lox* to produce marker-free GMO, referred as clean-gene technologies, could represents a suitable compromise between human and environmental safety-fears (Darbani *et al.,* 2007). Tomato is a crop of high economic interest, object of numerous studies and several breeding programs finalized to obtain improved varieties with better yield and quality. Both characters are severly influenced by environmental constraints, among which, biotic stresses have a key role. For this reason the study of plant-insect and plant-pathogen interactions is so central for the development of an environment-compatible agriculture. Plant defence to different damaging agents includes a myriad of complex and interconnected responses able to recognize and to act specifically against pests showing various modes of feeding and infection (Walling, 2009). Plant responses to herbivores are similar to those induced by wounding due to the mechanical damage that both cause, but they are not

completely overlapping. While injury-associated signals are important in plant–herbivore interactions, herbivore elicitors and effectors profoundly influence the specificity and magnitude, as well as the timing and spatial distribution, of induced defences (Walling, 2009). Herbivore-associated molecular patterns (HAMPs) and pathogen-associated molecular patterns (PAMPs) are recognized by plant receptors triggering induced defences. While PAMPs have been widely characterized, until now “true” HAMPs has still to be identified. Several insect elicitors and, recently, also volicitin and inceptin receptors have been characterized (Schmelz *et al.,* 2009). These compounds are not necessary for insect life, such as PAMPs for pathogens, so it raises some doubts about their definition as HAMPs. It is still not clear if PAMPs and HAMPs share common receptors and in positive case, which one is the step determining the specific responses activated by these different stressors. Calcium and calcium-sensor proteins, such as calmodulin and calcineurin-like, could interpret the recognition message. Not only calcium concentration, but also its spatial and temporal information have been associated to the transmission of a specific signal in both plant and animals (Luan *et al.,* 2002). Calcium and oxidases are good candidate to be effectors of hemipteran-induced defence responses. Plant responses to phloem-feeding insects share similarity to those induced by chewing insects and by pathogens. The similarity between plant responses to phloem-feeders, such as aphids, and pathogens is probably due to the similarity of aphid stylet and fugal hyphae penetrations (Walling, 2000). Like pathogens, insects have evolved strategies to evade plant defences and to successfully colonize the host plant. Transcriptomic and proteomic approaches spreading during the last decade have been providing a powerful push in the investigations on plant interactions with other organisms (Couldridge *et al.,* 2007; Kusnierczyc *et al.,* 2008; Li *et al.,* 2008; Chen *et al.,* 2010). Thanks to the emerging projects on genome sequencing and the spreading of “omics” disciplines, new elicitors, effectors and players of plant defence responses have been identified.

In tomato, systemin is the primary signal able to activate the cascade of reactions in response to wounding and chewing insects. Systemin has been widely characterized in its direct (McGurl *et al.,* 1992; McGurl *et al.,* 1994; Ryan and Pearce, 1998; Ryan, 2000; Schaller, 2009) and indirect (Corrado *et al.,* 2007; Degenhardt *et al.,* 2010) inducible tomato defence in responses to wounding and

chewing insects. Experiments with ProSys over-expressing plants by McGurl and collaborators (1994) demonstrated its activity in the up-regulation of proteinase inhibitors genes. Besides, ProSys involvment in PI induction, was confirmed by *in vivo* bioassay carried out with *Manduca sexta* larvae on tomato plants transformed with a *ProSys* antisense construct on which larval weight resulted higher than that registered for larvae fed on contro plants (Orozco-Cardenas *et al.,* 1993). Recently new peptides described by Narvaez-Vasquez and collaborators (2007) have been associated to systemin in the coordination of tomato defence responses in tomato and in Arabidopsis a systemin-functional homolog have been idendtified (Sun *et al.,* 2011). The important role of systemin and its precursor in the early steps of signal transduction and its role in the enhancement of parasitoid attractiveness (Corrado *et al.,* 2007) as well as salt stress tolerance (Orsini *et al.,* 2010) advanced the hypothesis that the peptide has a broad spectrum of activity in tomato stress responses. This study aimed to understand systemin activity in the molecular response of tomato plants defences activated by aphids and phytopatogenic fungi. To address this objective transgenic tomato plants over-expressing prosystemin gene were produced. The selection of the genotype to submit to genetic transformation was carefully evaluated. In fact most tomato cultivars harbour resistances to *Fusarium spp*, *Verticillium spp, Meloidogyne spp* and Tobacco Mosaic virus. These resistances are indicated as VFNT and are due to the presence of several resistance genes. In particular, the resistance to the nematode *Meloidogyne incognita* and three other species of root-knot nematode is attributed to *Mi* gene that confers also resistance to the potato aphid *Macrosiphum euphorbiae* and to the whitefly *Bemisia tabaci* (Kaloshian *et al.,* 1995). *Mi* affects not only aphid longevity but also its reproduction rate (Kaloshian *et al.,* 1997, 2000). *Mi* gene was introduced into cultivated tomato*, Solanum lycopersicum*, from its wild relative *S. peruvianum* and all tomato cultivars carrying almost a dominant allele at this *locus* showed resistance to several nematode species, aphids and whiteflies. Tomato cv. 'Red Setter' lacks dominant alleles at known defence locus, *Mi* included. The *Mi status* in this cultivar was checked by the analysis of CAPS-REX1 marker associated to this resistance gene (Williamson *et al*., 1994) confirming the lacking of dominant alleles at *Mi* locus. This feature makes the 'Red Setter' cultivar suitable to evaluate ProSys effect in responses to aphids. In fact, *Mi*-mediated resistance is able to cover and hide the effect of other resistance

genes as demonstrated by Cooper and collaborators (2005). They investigated about JA effect on aphid performance using JA exogenous applications to activate the induced resistance in two near-isogenic tomato cultivars with and without *Mi*. In their study, JA applications did not significantly impact on the fecundity or survivorship of aphid grown on the resistant line while its effect was observed in the susceptible line suggesting that *Mi* role has a major role in tomato resistance to aphid. For this reason, the evaluation of the function of a target gene in aphid response requires a genetic background that does not carry this defence gene. *Solanum lycopersicum* cv. 'Red Setter' was genetically transformed via *A. tumefaciens* containing the pMZ vector carrying *35S*$^2$*:prosystemin*. Only unique transformation events were selected and used for the generation of RSYS plants. A wide population of transgenic plants expressing *ProSys* at different levels was obtained. Different levels of transgene expression could be explained referring to the position effect due to the transgene random insertion within the plant genome caused by the agrobacterium-mediated transformation. Among $T_0$ population three genotypes were selected, for further investigations, according to their *ProSys* expression levels: RSYS24, the genotype expressing *ProSys*, at very high level, RSYS32, which shows an intermediate *ProSys* expression and RSYS17, a co-suppressed genotype. In order to check the *ProSys*-induced signalling cascade, the expression analysis of late defence genes located downstream the octadecanoid pathway was performed. Three genes coding for proteinase inhibitors (*InhI, InhII* and *MCPI*) were induced by *ProSys* over-expression providing a result consistent with literature (McGurl *et al.,* 1994; Ryan, 2000). A correlation between *ProSys* and *PIs* expression levels is hypothesized but further investigations on a larger number of replicates are needed to assess a significant quantitative relationship between *ProSys* and *PIs* expression levels and to verify if this quantitative effect is reflected during the steps of octadecanoid pathway. The production of a transgenic plant population showing different *PIs* expression levels allows to choose those lines producing amounts of PIs (~1% of total soluble proteins) conferring resistance to insects (Abdeen *et al.,* 2004) not too much compromised in their growth and production, since ProSys over-expressing plants have been associated to stunted phenotypes (McGurl *et al.,* 1994). These evaluations are very important in the development of engineered crops showing a not-altered yield production. This strategy is considered more efficient than the

combination of several PIs to overcome insects compensatory strategy (Abdeen *et al.,* 2004). These different *PIs* expression levels are not related to a different effect on *Spodoptera littoralis* larvae weights. In fact, as shown by bioassay, larvae fed on RSYS24 and RSYS32 leaves were similarly affected in their nutrition since they showed reduced weights. Larvae fed on RSYS17 plants showed intermediate weights between the two other transgenic genotypes and the control. Since the co-suppression of *ProSys* in this genotype causes the down-regulation of several JA-regulated genes, understanding the basis of this effect was priority. The comparison between the expression analysis of *ProSys* and *InhI* genes on 'Red Setter', RSYS24 and RSYS17 plants after *Spodoptera littoralis* feeding helped to explain this finding and to shed more light on ProSys involvement in tomato systemic response. *S. littoralis* feeding on 'Red Setter' plants induces only locally *ProSys*, while *InhI* is induced both locally and systemically. These data are consistent with the hypothesis by Lee and Howe (2003) that Sys locally induces JA synthesis to reach a threshold required for the activation of the systemic response. A systemin-independent *PIs* induction could be correlated to the weak affection of *Spodoptera littoralis* larvae weight observed on the co-suppressed RSYS17 plants by bioassay. In fact, insect chewing caused only a temporary induction of *ProSys* at 30 min after feeding. This result could be explained referring to the "RNA threshold" theory (Taylor, 1997). Referring to this model, RSYS17 could be a genotype in which the very high amount of *ProSys* transcript, possibly due to a high transgene copy number, could reach the threshold determining co-suppression. *Spodoptera littoralis* feeding could induce a feedback control of *ProSys* expression, decreasing temporary its transcript level and consequently its degradation. So, only for a short period of time, *ProSys* results to be induced by larvae elicitation due to a probable balance between transcript amount and threshold-dependent RNA interference activation. Despite the lack of *ProSys* induction, *InhI* was up-regulated in both damaged and undamaged leaves from 3 to 24 hours after feeding. Similar results were obtained on RSYS24, in which insect feeding didn't cause either increase or decrease of *ProSys* expression level. This is possibly the consequence of a feedback regulation exerted on prosystemin transcription, since the recent identification of JA-responsive elements in *ProSys* promoter (Avilés-Arnaunt and Délano-Frier, 2011). Interestingly, despite the high level of *PIs* constitutively expressed by these plants,

*Spodoptera* feeding further increased *InhI* expression in both local and distal leaves. *InhI* up-regulation in damaged leaves of highly *ProSys*-expression or *ProSys*-suppression could be explained by the release of plant elicitors (OGAs) or insect elicitors during herbivory. A basal systemic *PIs* expression in tomato *spr1* mutants that are impaired in the systemic response was also reported by Lee and Howe (2003). Spr1 influences systemin perception or a subsequent systemin-specific signalling event necessary for activation of the octadecanoid pathway (Lee and Howe, 2003). They found that *spr1* plants exhibit a low but significant level of wound-induced systemic *PIs* expression probably due to the existence of a systemin-independent wound response pathway. They supported this hypothesis by the observation that *spr1* plants were not affected in the expression of early wound-response genes such as *LoxD* and *AOS1*. Therefore systemic induction of *PIs* in RSYS17 could be attributed to a systemin-independent pathway. In RSYS24 the strong anti-herbivore activity could be the sum of the systemin signalling cascade and this independent one that both converge in the activation of JA pathway. The independent pathway could be induced by elicitors present in insect oral secretions or by plant elicitors, like OGAs, but it could be also mediated by the recently discovered hydroxyproline-rich systemin glycopeptides (HypSys) associated to ProSys in the coordination of tomato defence responses (Narvaez-Vasquez *et al.,* 2005). These molecules or new ones could locally trigger the activation of octadecanoid pathway stimulating JA production that reach a threshold required for the systemic response, similarly and independently to systemin activity. JA, or its derivated molecule JA-Ile, is then transported through the plant to activate defence genes in unwounded leaves and it is released to alert neighbours plants. Additional insights into the understanding of early signals responsible of the activation of long-distance response will evaluate the basis of this hypothesis.

ProSys has been recently associated to the promotion of tomato indirect defences acting in the modification of volatile blend released (Degenhardt *et al.,* 2010) and the consequent attraction of natural enemies of insect herbivores (Corrado *et al.,* 2007). Since *ProSys* over-expressing plants were found to be more attractive to the aphid parasitoid *Aphidius ervi* (Corrado *et al.,* 2007), its involvement in the regulation of direct defence in response to aphids is supposed. ProSys conferred-resistance to aphids and fungi evinced in bioassays underlining its broad-spectrum

role in tomato. A reduced aphid longevity and weight on RSYS24 plants were observed. Interestingly, weights of aphids fed on RSYS17 plants were higher than the control confirming that *ProSys* over-expression clearly affects aphid performance in tomato. These findings support the hypothesis that aphids avoid JA-related host defences by the promotion of SA pathway taking advantage of the antagonism between JA and SA pathways, firstly proposed for plant-whitefly interactions (Zarate *et al.,* 2007) and then extended also to plant-aphid interactions (Zhu-Salzman and Liu, 2005; De Vos *et al.,* 2007; Giordanengo *et al.,* 2010). The evaluation of possible interactions and maybe synergistic effects between *ProSys* and other defence genes, such as *Mi*, is an interesting field. The increased terpenoids release of *ProSys* over-expressing plants (Corrado *et al.,* 2007) also supports ProSys affection of aphid performance since terpenoid pathway was associated to the wheat resistance to the *Russian Wheat Aphid* (RWA) (Smith *et al.,* 2010). The molecular bases of RSYS resistance were investigated through a time-course expression analysis of genes involed in JA- and SA-pathways on 'Red Setter' untransformed plants after *M. euphorbiae* infestation. A strong induction of *InhI, Kunitz, Lap* and *Threonine deaminase* for JA pathway and *PR1* and WRKY for SA pathway was observed just 24 hours after aphid infestation. The maximum expression of these genes was observed 96 hours after aphid piercing. These data are consistent with recent findings by Li and collaborators (2006) about the induction of both SA and JA pathways in tomato after aphid infestation. Interestingly, *Threonine deaminase* that has been specifically associated to responses against lepidopteran herbivores (Vigil *et al.,* 2010) is induced by aphid feeding on tomato 'Red Setter' plants. The analysis of the same genes in RSYS24 and RSYS17 plants compared to the control revealed that they are induced by *ProSys* over-expression while are down-regulated in RSYS17 co-suppressed plants. As a consequence, RSYS24 and RSYS17 genotypes are two suitable models to study the involvement of JA and SA in tomato responses to aphids. The expression analysis on RSYS24 confirmed the attribution of the strong resistance to *M. euphorbiae* to JA pathway. Interestingly, *PR1* which is marker of the SAR (Walling, 2009), is strongly down-regulated by *ProSys* over-expression demonstrating that this gene is not relevant for resistance to aphids. Conversely, *PR1* is up-regulated in RSYS17 probably due to the down-regulation of JA pathway. Since RSYS17 didn't show effects on aphid performance, these support

the hypothesis that the aphid-induced SA defences are a strategy to avoid effective JA-regulated defences (Walling, 2008). The microarray analysis underlined many differentially expressed genes that explain these dual activation of JA and SA pathways. Moreover several new genes could be associated to the ProSys-modulation of multiple responses in tomato. First of all several genes coding for messengers involved in the early steps of stress responses are induced, such as *calmodulin 1*, *MAP kinase 3 (MPK3), calcium-dependent protein kinase (CDPK), Jasmonate-ZIM domain 3, purple acid phosphatises* and *LRR-domain receptor kinases.* The activation of so many signals could pre-alert these plants in the rapid activation of defence responses. Most of the genes involved in early and late steps of JA pathway are induced as well as several class of PR proteins and SA-regulated genes consistent with recent microarray dataset about Arabidopsis responses to the cabbage aphid *Brevicoryne brassicae* (Kusnierczyc *et al.,* 2008). Transcriptional studies of *Arabidopsis thaliana* infested by *Brevicoryne brassicae* showed up-regulation of several genes encoding proteins involved in ROS detoxification (Giordanengo *et al.,* 2010). According to these and other previous observations, *ProSys* over-expression up-regulates a large group of genes involved in oxidative stress such as NADH reductase, gluthadione-S-transferase, peroxidases, hydrogen peroxide-induced protein and tioredoxin. Several auxin-related transcripts that were up-regulated in RSYS samples may trigger the increase of ROS production, as demonstrated by Boyko and collaborators (2006) and Kawano (2003). Sugar transporters should also play an important role in aphid feeding, since their up-regulation during aphid infestation (Moran *et al.,* 2002; Divol *et al.,* 2005). They have been recently associated to the beneficial interactions between tomato and mychorrizae (Garcia-Rodriguez *et al.,* 2005). In this paper a characterization of the tomato *LeST3* sugar transporter was performed showing similarity with the *Arabidopsis ERD6* gene, a member of ERD family that in this research project have been linked to ProSys signalling through the analysis of protein-protein interactions. *LeST3* gene is regulated by mychorrizae in order to get nutrients (Garcia-Rodriguez *et al.,* 2005). The importance of these molecules in plant-aphid interactions is also demonstrated by the identification of sugar transporters of the major facilitator superfamily proteins in the pea aphid *Acyrthosiphon pisum* by Price workgroup (2010). The up-regulation of genes coding for sugar, aminoacids and nitrate transporters is

observed in RSYS plants, suggesting an higher mobilization of nutrients probably due to metabolism alterations caused by the constitutive activation of defence response of which aphid could profit. This findings suggest that RSYS plants could represent a better location for aphid development, but the observed reduced longevity and weight indicate that the antibiosis effect of ProSys-induced defence compounds is prevalent. Divol and collaborators (2005) reported the up-regulation of wall-associated enzymes after *Mizus persicae* infestation on *Apium graveolens*. The expression of these defence components were modulated in RSYS plants showing up-regulation of pectin methylesterase and several other enzymes involved in the phenylpropanoid pathway. This pathway is responsible of the production of many anti-microbial and anti-fungal compounds and is required for cell wall reinforcement (Naoumkina *et al.,* 2010). ProSys induction of phenylalanine ammonia lyase (PAL), caffeic acid O-methyltransferase (CAOMT) and ferulic acid hydroxylase (FAH), caffeoyl-CoA Omethyltransferase (CCoAMT) shares similarity with results previously described (Bhuiyan *et al.,* 2009) about wheat defence to powdery mildew. Another common feature with responses to pathogens is the induction of JA and ET pathways that act often synergistically (Bari and Jones, 2009). Most of the cited differentially expressed genes and pathways interested are also related to the observed resistance against *Botrytis cinerea*. Smaller necrosis areas and their slower development on transgenic *ProSys* over-expressing genotypes demonstrated the involvement of systemin in resistance against necrotrophic fungi. The most obvious explanation is the control exerted by ProSys on many JA-related genes, since necrotrophic fungi are more sensible to JA pathway (Walling, 2009). An important role in the resistance to pathogens is also played by other defence components such as LRR receptors, that are involved in PAMPs recognition (Wu and Baldwin, 2010), and enzymes involved in the oxidative burst. Besides, several class of PR proteins, such as PR2, PR5 and PR10 were strongly induced in RSYS plants. These induced molecules are the main players of SAR supportin a role of ProSys also on biotrophic fungi. As mentioned above, some SA-dependent genes are down-regulated by *ProSys* over-expression, while others are induced such as the pre-cited PR proteins. This could be the result of the balance between JA and SA concentrations that differentially influences their controlled pathways (Walling, 2009). The up-regulation of *GH3* supports this hypothesis since it is part of a large

family proteins that are early responsive auxin elements involved in the crosstalk between SA and auxins (Hagen *et al.,* 1985). Zhang and collaborators (2008) using double mutants *gh3/npr1* established that GH3 enhances the SA-mediated defence response through both *NPR1*-dependent and independent pathways. The GH3 and PR proteins induction require more investigations about ProSys involvement in responses to biotrophic fungi. A time-course expression analysis of *Botrytis*-responsive genes was carried out on 'Red Setter' plants after *Botrytis* inoculums in order to understand the hormonal regulation of the observed resistance. Early induced genes are *osmotin, extensin, miraculin, Pti5* and *arginase* and 96 hours post-inoculi also *InhI* is induced. Also in this case, JA- and SA-regulated genes are induced by *Botrytis* in tomato. These results were consistent with recent microarray datasets obtained on *Botrytis*-infected tomato (Asselbergh *et al.,* 2001) and were compared with the relative quantification of the same genes on RSYS24 and RSYS17 plants. By this comparison, the resistance to *Botrytis* in tomato could be divided into two components: the first controlled by SA and the second by JA. RSYS17 resistance to this necrotrophic fungus is due to the up-regulation of *osmotin* and *miraculin* that pre-alert transgenic plants to *Botrytis* infection. Referring to the widely reported antagonism between JA and SA, *ProSys* co-suppression and the consequent down-regulation of JA-related genes activate SA-mediated pathway conferring to RSYS17 plants resistance to *Botrytis cinerea.* Oppositely, RSYS24 resistance is attributed to the up-regulation of *InhI, Pti5* and *arginase*. Since RSYS24 is more resistant than RSYS17 the *ProSys*-induced JA component of tomato defence against *Botrytis* is stronger and more relevant than the SA effect in responses to necrotrophic fungi. In general, SA is active against biotrophic pathogens, whereas JA is effective against necrotrophs, which benefit from host cell death (Grant and Lamb, 2006). SA and JA signalling pathways can be either antagonistic or synergistic depending on the balance between their concentrations. Probably, in RSYS plants the induction of octadecanoid pathway produces an amount of JA such that SA is only partially antagonized, since the induction of some PR proteins. *ProSys* enhanced resistance against *Botrytis* could be also linked to its ability in the induction of terpenoids release (Corrado *et al.,* 2007) that have been recently associated to resistance to *Botrytis* in tomato (He *et al.,* 2006) and to the reduction of fungal hyphae length (Zhang *et al.,* 2008). Moreover, Liu and collaborators (2008)

recently tested the antifungal activities of nine fatty acids against four phytopathogenic fungi: *Alternaria solani, Colletotrichum lagenarium, Fusarium oxysporum f. sp. Cucumerinum, and Fusarium oxysporum f. sp. lycopersici*. The tested fatty acids were observed to inhibit the mycelial growth of the tested fungi. In their experiment they included linolenic acid that is released from plasma membrane by the systemin-induced phospholipase A2. The pathway analysis helped to understand at which phase of plant response ProSys activity is relevant to enhance tolerance. In a plant-pathogen interaction schematization 4 proteins coded by *ProSys* up-regulated genes were included and they were all associated to signal transduction and among them one is a PAMP receptor. This pathway analysis underlines the centrality of ProSys in the early steps of plant-pathogen interactions providing plants an optimal strategy for a very fast response.

Another interesting group of differentially regulated sequences have been associated to abiotic stress responses supporting recent findings from our lab (Orsini *et al.,* 2010). In this work a partial stomatal closure and a reduced growth in absence of salt stress in *ProSys* over-expressing tomato plants was linked to the high salt stress tolerance observed. The molecular bases of this resistance conferred by ProSys were attributed to the systemin-induced catalase (CAT1). The microarray analysis underlined various *ProSys* up-regulated sequences coding for hydrogen peroxide-induced proteins supporting these findings. Moreover, many other sequences were associated to abiotic stress responses, for example ultraviolet hypersensitive 3 (UVH3), several class of pathogenesis-related proteins, annexin 3-11 that have been associated to drought stress responses (Clark *et al.,* 2010), low temperature and salt responsive elements, late embryogenesis abundant proteins that are involved in lignifications and salt stress tolerance in potato (Park *et al.,* 2011), the GH3-like protein and microtubule-associated protein. The pathway analysis carried out with Paintomics underlined several enzymes coded by ProSys-regulated genes in the arginine and proline biosynthesis. Polyamines (putrescine, sperimine, spermidine) are compatible solutes produced by this pathway that have been widely investigated through transgenic approaches about their attitude to confer plants resistance to abiotic stresses (Hussein *et al.,* 2011). The enhancement of polyamines (PAs) biosynthesis reflects its effect also on biotic stress responses since PAs may be related to their multi-faceted nature, which includes working as an antioxidant, a free radical scavenger and a

membrane stabilizer (Larher *et al.,* 2003). The overlap with biotic responses is at the beginning steps of PAs biosynthesis since S-adenosyl-L-methionine is one of PAs precursor, common with ET biosynthesis. Not surprising, PAs are involved in programmed cell death (Zhao and Yang, 2008). Few reports recently indicated that PAs may act as cellular signals in intrinsic talk with hormonal pathways including ABA (Alcázar *et al.,* 2010a,b; Gill and Tuteja, 2010). So, polyamines activation by *ProSys* over-expression is another element characterizing the broad-spectrum activity of ProSys in the modulation of tomato responses to different stresses. As expected for a transgene insertion with a constitutive expression, the impact on metabolism is very strong as indicated by the annotation of many sequences in primary and secondary metabolisms. Consistent with previous phenotypic observations of BBS plants by McGurl and collaborators (1994), RSYS plants showed a stunted phenotype probably due to the high energetic demand for the continuous production of defensive compounds. This hypothesis was supported by Corrado and collaborators (2011) investigations about plant fitness affection by *ProSys* over-expression. They observed that *ProSys* constitutive expression reduced the number of seeds per fruit but they didn't found differences in fruit number, mass and germination percentages. These differences were attributed to constitutive defence costs due to the continuous production of defence compounds. The microarray analysis carried out on the RSYS transgenic lines allow to extend the attribution of this stunted phenotype also to the down-regulation of key genes involved in gibberellins biosynthesis that are known to be involved in internodes elongation. These evidences suggest a possible hormonal participation in control of the reduced size of *ProSys* over-expressing plants in combination with the high energetic request due to the constitutive activation of defence responses.

All these findings were strongly enriched in their power of knowledge thanks to the study of protein-protein interactions. Protein-protein interactions (PPIs) play a central role in all cell activities: structural function in the organization of organelles, transport within different sub-cellular compartments, signalling cascades activated in response to stimulus, regulation of gene expression, protein modification and many other processes. The production and the proper use of this type of information is of crucial importance in order to understand cell behaviour and, in the case of study, cell organization during defence responses. The availability of

PPIs data has been increasing enormously in the last few years with the emergence of high-throughput technologies that can report thousands of PPIs in a very short time. The aim of these techniques is to obtain a map describing all PPIs that can potentially occur in a cell that is defined “Interactome”. The PPIs analysis improves the information level obtained through the microarray analysis collocating predicted RSYS differentially expressed proteins in the network of plant proteins involved in the regulation of defence responses. This is a way to get a wider overview of ProSys impact on tomato transcriptome through the prediction of the interactions between proteins coded by the ProSys differentially expressed sequences. Moreover, the players of the correlation between different hormone-regulated pathways can be identified. Since a tomato interactome is not yet available, the shifting to the *Arabidopsis* interactome was necessary and let to improve the annotation level for the tomato differentially expressed genes. 695 differentially expressed sequences, corresponding to 485 unique ESTs were converted in their nearest Arabidopsis homologous protein as indicated by Meir and collaborators (2010) in order to get only the most similar sequences. ProSys homologous sequence has not been found in *Arabidopsis*. Recently, Huffacker and collaborators (2006) reported the isolation and characterization of small peptides, AtPep, that are functionally homologous to systemin. *Arabidopsis* and tomato belong different families, *Brassicaceae* and *Solanaceae*, respectively, that are located in two distinct phylogenetic divisions of dicots which diverged 150 million years ago based on a classification proposed by Soltis and collaborators (2002). Both these species have been widely studied as models, but several aspects of plant development and defence are different between them, for example JA biosynthesis and signalling. The comparison between *Arabidopsis* JA mutants and their tomato correspondents underlined many differences about their fertility and their contribution in the production of trienoic fatty acid (Sun *et al.,* 2011). *Arabidopsis* shows difference from tomato also in the systemic response, since Koo and collaborators (2010) proposed a model in which JA synthesis in systemic leaves is modulated by a JA-indipendent uncharacterized mobile signal. Two genomic studies by Allen (2002) and Van der Hoeven and coll. (2002) compares gene contents from *Solanum lycopersicum, Glycine max, Medicago Tracantula* and *Arabidopsis thaliana*. Between 10 and 15% of the contigs from this three tested species have no detectable homolog in any of the *Arabidopsis*

databases. In particular, 1002 tomato ESTs (17%) didn't found correspondent homolog representing probable gene-loss events in the *Arabidopsis* genome. Interestingly, genes that encode two of the major classes of systemin and JA-induced defense proteins in tomato, PI-II and polyphenol oxidase, are absent in *Arabidopsis*. Moreover, other tomato missing genes code for metallocarboxypeptidase inhibitor, ornithine decarboxylase and extensin-like proteins (Allen, 2002; Van der Hoeven *et al.,* 2002). These comparisons between tomato and Arabidopsis genomes content could explain the reduced number of Arabidopsis homologous proteins with significant correspondence with the differentially ESTs found in RSYS samples. The absence of late defence genes in RSYS network is not crucial considering the aim of the PPIs analysis and considering that, as late defence gene products they are known to act against the insect/pathogen/stress factor, so generally they are not expected to show relevant interactions with other plant proteins. The RSYS network obtained with various bioinformatic tools contains 2066 proteins and among them 195 were from RSYS list showing a good representation of the differentially expressed sequences. This network was evaluated by statistical analysis compared to thousands random networks showing the same size. This analysis gave positive results since each coefficient under study in RSYS network was statistically higher than random networks revealing its high clustering attitude and underlined about 30 hubs, considering as hub nodes with a degree higher than 20 (Aragues *et al.,* 2007). The collapsing approach helped to simplify some areas of the network allowing the biological interpretation of several hubs. The highest connected node of RSYS network is PAP3 showing 275 interactions. Since it is coded by a ProSys up-regulated gene, the induction of its interactions within RSYS network are assumed to be promoted. This assumption lead the signal transduction component to be dominant within the network. In fact, in this node the connection between responses to different stressors can be easily seen finding signal molecules as mediators. PAP3 interacts with more than 30 classes of kinases and among them the wall-associated kinase (WAK1) is particularly interesting since it represents the link between the apoplast and cytoplasm. This is a good connector between different type of responses since it is involved in the pathogenesis (He *et al.,* 2006) and in tolerance to aluminum (Sivaguru *et al.,* 2003). FAB2 represents another polyvalent molecule since it is involved in responses to viruses, bacteria and

insects and in the crosstalk between JA and SA pathways. The interactions of the early responsive to dehydration protein (ERD4) coded by a ProSys-induced genes supports the previous dissertation about ProSys enhancement of responses against abiotic stresses. The interaction with several heat shock proteins and DNA-J chaperonine confirms that RSYS plants could also show tolerance to high or low temperatures. The interaction with DNA-J is a clear connection between abiotic and biotic stress responses, since it is induced by *Fusarium* (Boddu *et al.,* 2006) and by potyvirus (Alfenas-Zerbini *et al.,* 2009) infections. This connection is also read in ERD4 interaction with LRR-RLK (SRF), known to be involved in PAMP recognition. Interestingly, abiotic stress responses are also linked to volatile emissions as indicated by ERD4 interaction with farnesyl pyrophosphate synthase (FPS) involved in terpenoid biosynthesis. The direct interaction between these defence components could be mediated by ethylene (ET), since ET works in cooperation with JA in the modulation of defence responses and it is involved in the forefront of cell death. It's a long time that reduced nitrogen availability has been associated to the promotion of synthesis and accumulation of sesquiterpenes in leaves (Wander and Bouwmeester, 1998). More recently, Schmelz and collaborators (2003) examined the interaction of volicitin, JA, and ET on the induction of volatile emission at different levels of nitrogen (N) availability that are known to influence ET sensitivity. They found that in reduced N availability, ET synergized volicitin induced volatile emission in plant. So, in the ERD4 node a new link between environmental stresses and volatile release in underlined. The network analysis allowed to see the direct relationship between several families of transcription factors, known to regulate plant immune responses against pathogens and pests (Singh *et al.,* 2002), resulted differentially expressed by microarray. A fairly representative group of TFs were up-regulated in RSYS plants such as WRKY, bZIP, MYB, GRAS, TGA and Ap2Erf. All these TFs interacts with calmodulin 1 (CAM1), that could be defined as the best example of node with a polyvalent control in plant defence. Here the intersection between SA-, JA-, ABA- and auxin-regulated pathways is observed in addition to the adjacency of cytoskeleton components, enzymes involved in phenylpropanoid and brassinosteroid pathways and proteins involved in RNA interference that are other important elements of stress response regulation. $H_2O_2$ and cortical microtubule alterations have been correlated with the activation of defence response to

*Verticillium dhaliae* toxins (Yao *et al.,* 2011). Exploring RSYS network many interesting interactions between old and new characters of plant defence response are discovered allowing the connection between defence aspects before considered distant. This kind of analysis enlarged the knowledge of ProSys role in plant defence provided by the microarray finding further supports to the observed biological resistance of RSYS plants. The study of PPIs of ProSys-regulated proteins could be improved when a tomato interactome will be available since it will provide more information about tomato-specific responses.

This study demonstrated the central role of ProSys on tomato responses to several stressors, spreading from environmental ones to different pests. ProSys involvement in the early steps of stress perception promotes the activation of very fast responses, conferring to the plant a good ability to limit damages and in some cases, such as against necrotrophic fungi, to stop the attack. The transgenic plant population produced represents a very useful genetic material to evaluate a possible quantitative effect of ProSys in tomato and to get a greater insight on the crosstalk between different defence pathways. The introduction of systemin/JA mutants and reverse genetic approaches targeting new interesting genes underlined during this study could help to attribute new functions and to evaluate new players of tomato defence responses.

# 5. REFERENCES


**Abdeen, A., Virgos, A., Olivella, E., Villanueva, J., Aviles, X., Gabarra, R., and Prat, S.** (2005). Multiple insect resistance in transgenic tomato plants over-expressing two families of plant proteinase inhibitors. *Plant Molecular Biology*. **57**: 189-202.

**Adie B. A., Perez-Perez J.** (2007). ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defences in Arabidopsis. Plant Cell **19:**1665–1681

**Agrios G.N.** (2005). *Plant Pathology*, 5th edn. San Francisco, CA: Elsevier Academic Press

**Alborn H. T., Hansen T. V., Jones T. H., Bennett D. C., Tumlinson J. H**. (2007). Disulfooxy fatty acids from the American bird grasshopper Schistocerca americana, elicitors of plant volatiles. Proc. Natl. Acad. Sci. USA **104:**12976–81

**Alcázar R., Altabella T., Marco F., Bortolotti C., Reymond M., Knocz C., Carrasco P., Tiburcio A. F.** (2010a). Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. *Planta.* **231:**1237–49

**Alfenas-Zerbini P., Maia I. G., Fávaro R. D., Cascardo J. C. M., Brommonschenkel S. H. and Zerbini F. M.** (2009). Genome-Wide Analysis of Differentially Expressed Genes During the Early Stages of Tomato Infection by a Potyvirus. *MPMI* **22:** 352-361

**Allen K.D.** (2002). Assaying gene content in Arabidopsis. Proc. Natl Acad. Sci. U S A. **99:** 9568–9572

**Altenbach D., Robatzek S.** (2007). Pattern recognition receptors: From the cell surface to intracellular dynamics. *Molecular Plant-Microbe Interactions* **20:** 1031–1039

**Aoki K., Yano K., Suzuki A., Kawamura S., Sakurai N., Suda K., Kurabayashi A., Suzuki T., Tsugane T., Watanabe M., Ooga K., Torii M., Narita T., Shin-I T., Kohara Y., Yamamoto N., Takahashi H., Watanabe Y., Egusa M., Kodama M., Ichinose Y., Kikuchi M., Fukushima S., Okabe A., Arie T., Sato Y., Yazawa K., Satoh S., Omura T., Ezura H., Shibata D.** (2010). Large-scale analysis of full-length cDNAs from the tomato (Solanum lycopersicum) cultivar Micro-Tom, a reference system for the Solanaceae genomics. *BMC genomics.* **11:**210.

**Aragues R., Sali A., Bonet J., Marti-Renom M. A., Oliva B.** (2007). Characterization of protein hubs by inferring interacting motifs from protein interactions. *PLoS Comput Bio* **3:** e178

**Arimura G. I., Köpke S., Kunert M., Volpe V., David A., Brand P., Dabrowska P., Maffei M. E. and Boland W.** (2008). Effects of feeding *Spodoptera littoralis* on Lima bean leaves: IV. Diurnal and nocturnal damage differentially initiate plant volatile emission. *Plant Physiology* **146:** 965–973

**Asselbergh B, Curvers K, Franca SC, Audenaert K, Vuylsteke M, Van Breusegem F, Höfte M.** (2001). Resistance to Botrytis cinerea in sitiens, an abscisic acid-deficient tomato mutant, involves timely production of hydrogen peroxide and cell wall modifications in the epidermis. *Plant Physiol.* **144(4):**1863-77.

**Bari R., Jones J. D.** (2009). Role of plant hormones in plant defence responses. *Plant Mol Biol.* **69(4):**473-88

**Baumann P.** (2005). Biology of bacteriocyte-associated endosymbionts of plant sapsucking insects. *Annual Review of Microbiology* **59:** 155–189

**Bayés A., Comellas-Bigler M., Rodríguez de la Vega M., Maskos K., Bode W., Aviles F. X., Jongsma M. A., Beekwilder J., Vendrell J.** (2005). Structural basis of the resistance of an insect carboxypeptidase to plant protease inhibitors. *Proc Natl Acad Sci USA* **102(46):**16602-7

**Berenbaum M. R. and Zangerl A. R.** (2008). Facing the future of plant–insect interaction research: Le retour a la ‘‘raison d’être. *Plant Physiology* **146**: 804–811

**Besser K., Harper A., Welsby N.A., Schauvinhold I., Slocombe S.P., Li Y., Dixon R. A., Broun P.** (2007). Diverged regulation of terpenoid metabolism in the trichomes of a wild and cultivated tomato species. Unpublished.

**Bhuiyan N. H., Selvaraj G., Wei Y., King J.** (2009). Gene expression profiling and silencing reveal that monolignol biosynthesis plays a critical role in penetration defence in wheat against powdery mildew invasion. *J Exp Bot.* **60(2):**509-21

**Bhuiyan N. H., Selvaraj G., Wei Y., King J.** (2009). Gene expression profiling and silencing reveal that monolignol biosynthesis plays a critical role in penetration defence in wheat against powdery mildew invasion. *J Exp Bot.* **60(2):**509-21

**Birnboim H. C., Doly J.** (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **7(6):**1513-23.

**Boddu J., Cho S., Kruger W. M., Muehlbauer G. J.** (2006). Transcriptome analysis of the barley–*Fusarium graminearum* interaction. *Mol Plant Microbe Interact*. **19:**407–417

**Boudsocq M., Willmann M. R., McCormack M., Lee H., Shan L**. (2010). Differential innate immune signaling via $Ca^{2+}$ sensor protein kinases. *Nature* doi:10.1038/nature08794 Letter

**Bozzo G. G., Raghothama K. G., Plaxton W. C.** (2002). Purification and characterization of two secreted purple acid phosphatase isozymes from phosphate-starved tomato (*Lycopersicon esculentum*) cell cultures. *Eur. J. Biochem.* **269:** 6278–6286

**Bradford M.M.** (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry* **72:** 248-254

**Brodersen P., Petersen M., Bjørn Nielsen H., Zhu S., Newman M. A., Shokat K. M., Rietz S., Parker J., Mundy J.** (2006). Arabidopsis MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. *The Plant Journal* **47:** 532–546

**Bruinsma M., Van Dam N. M., Van Loon J. J. A., Dicke M.** (2007). Jasmonic acid induced changes in *Brassica oleracea* affect oviposition preference of two specialist herbivores. *J Chem Ecol* **33:** 655–668

**Chen H., Gonzales-Vigil E., Wilkerson C. G., Howe G. A.** (2007). Stability of plant defense proteins in the gut of insect herbivores. *Plant Physiology*. **143**: 1954-1967

**Chen H., Wilkerson C. G., Kuchar J. A., Phinney B. S., Howe G. A.** (2005). Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. *Proc Natl Acad Sci U S A*. **102(52):**19237-42

**Chilton, M.D., Currier, T.C., Farrand, S.K., Bendich, A.J., Gordon, M.P., e Nester, E.W.** (1974). Agrobacterium tumefaciens DNA and PS8 bacteriophage DNA not detected in crown gall tumors. Proc Natl Acad Sci U S A **71:** 3672-3676.

**Chen X., Niks R. E., Hedley R. E., Morris J., Druka A., Marcel T. C.** (2010). Differential gene expression in nearly isogenic lines with QTL for partial resistance to Puccinia hordei in barley. *BMC Genomics*. **11:** 629

**Chinchilla D., Zipfel C., Robatzek S. (2007).** A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature* **448:**497–500

**Chong J., Baltz R., Schmitt C., Beffa R., Fritig B., Saindrenan P.** (2002) Downregulation of a pathogen-responsive tobacco UDP-Glc: phenylpropanoid glucosyltransferase reduces scopoletin glucoside accumulation, enhances oxidative stress, and weakens virus resistance. *Plant Cell*, **14**: 1093–1107.

**Clark G., Konopka-Postupolska D., Hennig J., Roux S.** (2010). Is annexin 1 a multifunctional protein during stress responses? *Plant signal behaviour.* **5(3):**303-7.

**Consonni C., Humphry M. E., Hartmann H. A., Livaja M., Durner J., Westphal L., Vogel J., Lipka V., Kemmerling B., Schulze-Lefert P., Somerville S. C., Panstruga R.** (2006). Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nat Genet.* **38(6):**716-20

**Constabel C. P., Yip L., Ryan C. A.** (1998). Prosystemin from potato, black nightshade, and bell pepper: primary structure and biological activity of predicted systemin polypeptides. Plant Mol Biol **36:**55–62

**Cooper W.E.**, **Goggin F.L.** (2005). Effects of jasmonate-induced defenses in tomato on the potato aphid, *Macrosiphum euphorbiae*. *Entomologia Experimentalis et Applicata.* **115**: 107-115.

**Corrado G., Agrelli D., Rocco M., Basile B., Marra M. and Rao R.** (2011). Systemin-inducible defences against pests are costly in tomato. *Biologia Plantarum.* **55(2):** 305-311

**Corrado G.**, **Sasso R.**, **Pasquariello M.**, **Iodice L.**, **Carretta A.**, **Cascone P.**, **Ariati L.**, **Digilio M.C.**, **Guerrieri E.**, **Rao R.,** (2007). Systemin Regulates Both Systemic and Volatile Signaling in Tomato Plants. *Journal of Chemical Ecology* **4**: 669-681.

**Couldridge C., Newbury H. J., Ford-Lloyd B., Bale J., Pritchard J.** (2007). Exploring plant responses to aphid feeding using a full Arabidopsis microarray reveals a small number of genes with significantly altered expression. *Bulletin of Entomological Research*. **97:** 523–532

**Darbani B., Eimanifar A., Stewart C. N., Camargo W. N.** (2007). Methods to produce marker-free transgenic plants. *Biotechnol. J*. **2:** 83–90

**De Vos M., Van Oosten V. R., Van Poecke R. M. P., Van Pelt J. A., Pozo M. J., Mueller M. J., Buchala A. J., Metraux J.-P., Van Loon L. C., Dicke M.** (2005) Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. *Mol Plant Microbe Interact* **18:** 923–937

**Degenhardt D. C., Refi-Hind S., Stratmann J. W., Lincoln D. E.** (2010). Systemin and jasmonic acid regulate constitutive and herbivore-induced systemic volatile emissions in tomato, Solanum lycopersicum. *Phytochemistry.* **71(17-18):**2024-37

**Del Pozo J. C. , Allona I. , Rubio V. , Leyva A. , de la Peña, A., Aragoncillo, C.& Paz-Ares, J.**(1999) A type 5 acid phosphatase gene from Arabidopsis thaliana is induced by phosphate starvation and by some other types of phosphate mobilizing/oxidative stress conditions. *Plant J.* **19:** 579–589.

**Delano J.P., Dombrowski J.E., Ryan C.A.** (1999). The expression of tomato prosystemin in *Escherichia coli*: a structural challenge. *Protein Expression and Purification* **17:** 74-82

**Denoux C., Galletti R., Mammarella N., Gopalan S., Werck D., De Lorenzo G., Ferrari S., Ausubel F. M. and Dewdney J.** (2008). Activation of defense response pathways by OGs and Flg22 elicitors in Arabidopsis seedlings. *Molecular Plant* **1:** 423–445

**Deslandes L., Rivas S.** (2011). The plant cell nucleus A true arena for the fight between plants and pathogens. *Plant Signaling & Behavior* **6(1):** 42-48

**Dicke, M.** (1999). Specificity of herbivore-induced plant defenses. *Novartis Foundation Symposium***. 223:** 43-59.

**Dinant S., Clark A. M., Zhu Y., Vilaine F., Palauqui J. C., Kusiak C., Thompson G. A.** (2003). Diversity of the superfamily of phloem lectins (phloem protein 2) in angiosperms. *Plant Physiol.* **131(1):**114-28

**Ding X., Cao Y., Huang L., Zhao J., Xu C., Li X., Wang S.** (2008). Activation of the indole-3-acetic acid-amido synthetase GH3-8 suppresses expansin expression and promotes salicylate- and jasmonate-independentbasal immunity in rice. *Plant Cell* (Epub ahead of print).

**Dixon R.A., Achnine L., Kota P., Liu C.J., Reddy M.S.S., Wang L.** (2002). The phenylpropanoid pathway and plant defence—a genomics perspective. *Mol. Plant Pathol.* **3**: 371–390.

**Dóczi R., Brader G., Pettkó-Szandtner A., Rajh I., Djamei A., Pitzschke A., Teige M. and Hirt H.** (2007). The Arabidopsis mitogen-activated protein kinase kinase MKK3 is upstream of group C mitogen-activated protein kinases and participates in pathogen signaling. *The Plant Cell* **19:** 3266–3279

**Dong X.** (2004). NPR1, all things considered. *Curr Opin Plant Biol.* **7(5):**547-52

**Duff S.M.G., Sarath G., Plaxton W.C.** (1994). The role of acid phosphatases in plant phosphorus metabolism. *Physiol. Plant*. **90**: 791–800.

**Engelberth J.**, **Alborn H.T.**, **Schmelz E.A.**, **Tumlinson J.H.**, (2004). Airbone signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences, USA* **101**: 1781-85.

**Eyüboglu B., Pfister K., Haberer G., Chevalier D., Fuchs A., Mayer K. F., Schneitz K.** (2007). Molecular characterisation of the STRUBBELIG-RECEPTOR FAMILY of genes encoding putative leucine-rich repeat receptor-like kinases in Arabidopsis thaliana. *BMC Plant Biol*. **7:**16.

**Farmer E. E., Ryan C. A.** (1990). Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proc Natl Acad Sci U S A.* **87(19):**7713-6.

**Fatouros N. E., Broekgaarden C., Bukovinszkine'Kiss G., van Loon J. J., Mumm R.** (2008). Male-derived butterfly antiaphrodisiac mediates induced indirect plant defense. *Proc. Natl. Acad. Sci. USA* **105:**10033–38

**Felton G.W., Tumlinson J.H.** (2008) Plant–insect dialogs: complex interactions at the plant–insect interface. *Current Opinion in Plant Biology* **11**: 457–463.

**Ferrari S., Galletti R., Denoux C., De Lorenzo G., Ausubel F. M. and Dewdney J.** (2007). Resistance to Botrytis cinerea induced in Arabidopsis by elicitors is independent of salicylic acid, ethylene, or jasmonate signalling but requires PHYTOALEXIN DEFICIENT3. *Plant Physiology* **144:** 367–379

**Ferry N., Edwards M. G., Gatehouse J., Capell T., Christou P., Gatehouse A. M.** (2004). Transgenic plants for insect pest control: a forward looking scientific perspective. *Transgenic Res.* **15(1):**13-9.

**Flors V., Ton J., van Doorn R., Jakab G., García-Agustín P., Mauch-Mani B.** (2008). Interplay between JA, SA and ABA signalling during basal and induced resistance against Pseudomonas syringae and Alternaria brassicicola. *Plant Journal* **54(1):**81-92

**Fontes E. P. B., Santos A. A., Luz D. F., Waclawovsky A. J., Chory J.** (2004). The geminivirus NSP acts as virulence factor to suppress an innate transmembrane receptor kinase-mediated defense signaling. *Genes and Development* **18:** 2545–2556.

**Fowler J. H., Narváez-Vásquez J., Aromdee D. N., Pautot V., Holzer F. M., Walling L. L.** (2009). Leucine aminopeptidase regulates defense and wound signaling in tomato downstream of jasmonic acid. *Plant Cell.* **21(4):**1239-51

**Frost C. J., Mescher M. C., Carlson J. E. and De Moraes C. M.** (2008). Plant defense priming against herbivores: Getting ready for a different battle. *Plant Physiology* **146:** 818–824

**Fulton, T.M., Chunwongse, J., e Tanksley, S.D.** (1995). Microprep Protocol for Extraction of DNA from Tomato and other Herbaceous Plants. Plant Molecular Biology Reporter **13:** 207-209.

**Funk C. J.** (2001). Alkaline phosphatase activity in whitefly salivary glands and saliva. *Archives of Insect Biochemistry and Physiology* **46:** 165–174

**García-Rodríguez S., Pozo M. J., Azcón-Aguilar C., Ferrol N.** (2005). Expression of a tomato sugar transporter is increased in leaves of mycorrhizal or Phytophthora parasitica-infected plants. Mycorrhiza **15:** 489–496

gene in transgenic tomato plants generates a systemic signal that constitutively induces

**Genre A., Bonfante P.** (2002) Epidermal cells of a symbiosis defective mutant of *Lotus japonicus* show altered cytoskeleton organization in the presence of a mycorrhizal fungus. *Protoplasma.* **219:** 43–50.

**Gill S. S., Tuteja N.** (2010). Polyamines and abiotic stress tolerance in plants. Plant Signal Behav**. 51:**26–33

**Gilliham M., Dayod M., Hocking B. J., Xu B., Conn S. J., Kaiser B. N., Leigh R. A., Tyerman S. D.** (2011). Calcium delivery and storage in plant leaves: exploring the link with water flow. J Exp Bot. **62(7):**2233-50

**Giordanengo P., Brunissen L., Rusterucci C., Vincent C., van Bel A., Dinant S., Girousse C., Faucher M., Bonnemain J.-L.** (2010). Compatible plant-aphid interactions: How aphids manipulate plant responses. *C. R. Biologies* **333:** 516–523

**Giri A. P., Wunsche H., Mitra S., Zavala J. A., Muck A**. (2006). Molecular interactions between the specialist herbivore Manduca sexta (Lepidoptera, Sphingidae) and its natural host Nicotiana attenuata. VII. Changes in the plant's proteome. *Plant Physiol*. **142:**1621–41

**Gonzales-Vigil E., Bianchetti C. M., Phillips G. N. Jr, Howe G. A.** (2011). Adaptive evolution of threonine deaminase in plant defense against insect herbivores. *Proc Natl Acad Sci U S A.* **108(14):**5897-902

**Górecka K. M., Trebacz K., Górecki R., Pikula S.** (2007). Participation of annexin At1 in plant response to abiotic stress. *Postepy biochem* **53(2):** 154-8

**Grant M., Lamb C.** (2006). Systemic immunity. *Curr Opin Plant Biol.* **9(4):**414-20

**Green T. R., Ryan C. A.** (1972). Wound-induced proteinase inhibitor in plant leaves – possible defense mechanism against insects. *Science* **175:**776–777

**Gu Y. Q., Chao W. S., Walling L. L.** (1996). Localization and post-translational processing of the wound-induced leucine aminopeptidase proteins of tomato. *J Biol Chem.* **271(42):**25880-7.

**Habib H.**, **Fazili K. M.** (2007). Plant protease inhibitors: a defense strategy in plants. *Biotechnology and Molecular Biology review*. **2**(3), 068-085.

**Hagen G., Kleinschmidt A., Guilfoyle T.** (1984). Auxin-regulated gene expression in intact soybean hypocotyl and excised hypocotyls sections. Planta **162:** 147–153

**Hatchez C., Chaumont F.** (2010). Aquaporins: a family of highly regulated multifunctional channels. *Adv Exp Med Biol.* **679**:1-17.

**He P.Q., Tian L., Chen K. S., Hao L. H., Li G. Y.** (2006). Induction of Volatile Organic Compounds of Lycopersicon esculentum Mill. and Its Resistance to Botrytis cinerea Pers. by Burdock Oligosaccharide. *Journal of Integrative Plant Biology* **48**: 550-557.

**He Z. H., Fujiki M., Kohorn B. D.** (1996). A cell wall-associated, receptor-like protein kinase. *J Biol Chem* **271:** 19789–19793

**Heitz T., Bergey D. R., Ryan C.A.** (1997). A gene encoding a chloroplast-targeted lipoxygenase in tomato leaves is transiently induced by wounding, systemin, and methyl jasmonate. *Plant Physiol.* **114(3):**1085-93.

**Hilker M., Meiners T.** (2006) Early herbivore alert: insect eggs induce plant defense. *Journal Chemical Ecology* **32**: 1379–1397

**Hou X., Tong H., Selby J., Dewitt J., Peng X,. He Z. H.** (2005). Involvement of a cell wall-associated kinase, WAKL4, in Arabidopsis mineral responses. *Plant Physiol.* **139(4):**1704-16

**Howe G. A. and Jander G.** (2008). Plant immunity to insect herbivores. *Annual Review of Plant Biology* **59:** 41–66

**Huffaker A., Pearce G., and Ryan C.A.** (2006). An endogenous peptide signal in Arabidopsis activates components of the innate immune response. Proc. Natl Acad. Sci. U S A. **103:** 10098–10103

**Hunt A. G.** (1988). Identification and characterization of cryptic polyadenylation sites in the 3' region of a pea ribulose-1,5-bisphosphate carboxylase small subunit gene. *DNA*. **7(5):**329-36

**Hussain S. S., Ali M., Ahmad M., Siddique K. H. M.** (2011). Polyamines: Natural and engineered abiotic and biotic stress tolerance in plants. *Biotechnology Advances* **29:** 300–311

inhibition of the systemin precursor gene. *Science* **255:**1570–1573

**Jakob K., Kniskern J. M., Bergelson J.** (2007). The role of pectate lyase and the jasmonic acid defense response in Pseudomonas viridiflava virulence. *Mol Plant Microbe Interact*. **20(2):**146-58.

**Jensen K. M., Kjaersgaard T., Nielsen M. M., Galberg P., Petersen K., O'Shea C., Skriver K.** (2010). The Arabidopsis thaliana NAC transcription factor family: structure–function relationships and determinants of ANAC019 stress signalling. *Biochem. J.* **426**: 183–196

**Jones J. D. G., Dangl J. L.** (2006). The plant immune system. *Nature* **444**: 323–329

**Jones J. D. G.** (2011). Why genetically modified crops? *Phil. Trans. R. Soc. A* **369:** 1807-1816

**Joo S., Liu Y., Lueth A., Zhang S.** (2008). MAPK phosphorylation-induced stabilization of ACS6 protein is mediated by the non-catalytic C-terminal domain, which also contains the cis-determinant for rapid degradation by the 26S proteasome pathway. *Plant Journal* **54(1):**129-40

**Jordá L., Coego A., Conejero V., Vera P.** (1999). A genomic cluster containing four differentially regulated subtilisin-like processing protease genes is in tomato plants. *J Biol Chem.* **274(4):**2360-5

**Kaku H., Nishizawa Y., Ishii-Minami N., Akimoto-Tomiyama C., Dohmae N., Takio K., Minami E. and Shibuya N.** (2006). Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 11086–11091

**Kaloshian I.**, **Kinsey M.G.**, **Williamson V.M.**, **Ullman D.E.** (2000). *Mi*-mediated resistance against the potato aphid *Macrosiphum euphorbiae* (Hemiptera: Aphididae) limits sieve element ingestion. *Environmental Entomology* **29**: 690-695

**Kaloshian I., Lange W. H., Williamson V. M.** (1995). An aphid-resistance locus is tightly linked to the nematode-resistance gene, Mi, in tomato. *Proc Natl Acad Sci U S A*. **92(2):**622-5.

**Kaloshian I., Yaghoobi J., Liharska T., Hontelez J., Hanson D., Hogan P., Jesse T., Wijbrandi J., Simons G., Vos P., Zabel P., Williamson V. M.** (1998). Genetic and physical localization of the root-knot nematode resistance locus mi in tomato. *Mol Gen Genet*. **257(3):**376-85

**Kandoth P. K., Ranf S., Pancholi S. S., Jayanty S., Walla M. D., Miller W., Howe G. A., Lincoln D. E. and Stratmann J. W.** (2007). Tomato MAPKs LeMPK1, LeMPK2, and LeMPK3 function in the systemin-mediated defense response against herbivorous insects. *Proceedings of the National Academy of Sciences of the United States of America* **104:** 12205–12210

**Kawano T.** (2003). Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction. *Plant Cell Reports* **21:** 829–837

**Kehr J.** (2006). Phloem sap proteins: their identities and potential roles in the interaction between plants and phloem-feeding insects. *Journal of Experimental Botany*. **57(4):** 767–774

**Kempema L. A., Cui X., Holzer F. M. and Walling, L. L.** (2007). Arabidopsis transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids. *Plant Physiology* **143:** 849–865

**Kesarwani M., Yoo J., Dong X.** (2007). Genetic interactions of TGA transcription factors in the regulation of pathogenesis-related genes and disease resistance in Arabidopsis. *Plant physiology.* **144(1):** 336-46

**Kessler A., Baldwin I.T.** (2002). Plant responses to insect herbivory: the emerging molecular analysis. *Annual Review of Plant Biology* **53:** 299-328.

**Kessler A., Halitschke R., Diezel C., Baldwin I. T.** (2006). Priming of plant defense responses in nature by airborne signalling between *Artemisia tridentata* and *Nicotiana attenuata. Oecologia* **148:** 280-292

**Kim J. S., Mizoi J., Yoshida T., Fujita Y., Nakajima J., Ohori T., Todaka D., Nakashima K., Hirayama T., Shinozaki K., Yamaguchi-Shinozaki K.** (2011). An ABRE promoter sequence is involved in osmotic stress-responsive expression of the DREB2A gene, which encodes a transcription factor regulating drought-inducible genes in Arabidopsis. *Plant Cell Physiol*

**Kitsios and Doonan.** (2011). Cyclin dependent protein kinases and stress responses in plants. *Plant Signaling & Behavior* . **6(2):** 204-209

**Kliebenstein D. J. and Rowe H. C.** (2008). Ecological costs of biotrophic versus necrotrophic pathogen resistance, the hypersensitive response and signal transduction. *Plant Science* **174:** 551–556.

**Kobayashi M., Ohura I., Kawakita K., Yokota N., Fujiwara M.** (2007). Calcium-dependent protein kinases regulate the production of reactive oxygen species by potato NADPH oxidase. *Plant Cell* **19:**1065–80

**Konno K.** (2011). Plant latex and other exudates as plant defense system: roles of various defense chemicals and proteins contained therein. *Phytochemistry* **72:** 1510-1530

**Koo A. J., Gao X., Jones A. D., Howe G. A.** (2009). A rapid wound signal activates the systemic synthesis of bioactive jasmonates in *Arabidopsis. Plant J.* **59:**974–86

**Kruzmane D., Jankevica L., Ievinsh G.** (2002). Effect of regurgitant from *Leptinotarsa decemlineata* on wound responses in *Solanum tuberosum* and *Phaseolus vulgaris*. *Physiol. Plantarum.* **115:**577–84

**Kusńierczyk A., Winge P., Jørstad T. S., Troczyńska J., Rossiter J. T. and Bones A. M.** (2008). Towards global understanding of plant defence against aphids-timing and dynamics of early *Arabidopsis* defence responses to cabbage aphid (*Brevicoryne brassicae*) attack. Plant, Cell & Environment **31:** 1097–1115

**Kuśnierczyk A., Winge P., Midelfart H., Armbruster W. S., Rossiter J. T., Bones A. M.** (2007). Transcriptional responses of *Arabidopsis thaliana* ecotypes with different glucosinolate profiles after attack by polyphagous *Myzus persicae* and oligophagous *Brevicoryne brassicae. Journal of Experimental Botany* **58:** 2537–2552

**Laemmi U. K.** (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227(5259):**680-5

**Lannoo N., Van Damme E.J.M.** (2010). Nucleocytoplasmic plant lectins. *Biochim. Biophys. Acta* **1800:** 190–201.

**Larher F. R., Aziz A., Gibon Y., Trotel-Aziz P., Sulpice R., Bouchereau A.** (2003). An assessment of the physiological properties of the so-called compatible solutes using in vitro experiments with leaf discs. *Plant Physiol Biochem* **41:**657–66

**Lee G.I., and Howe G.A.** (2003). The tomato mutant spr1 is defective in systemin perception and the production of a systemic wound signal for defense gene expression. Plant J. **33:** 567–576

**Li L., Li C., Lee G.I., Howe G.A.** (2002). Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato. Proc. Natl Acad. Sci. U S A. **99:** 6416–6421

**Li L., Zhao Y., McCaig B.C., Wingerd B.A., Wang J., Whalon M.E., Pichersky E., Howe G.A.** (2004). The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *Plant Cell.* **16:** 126-143

**Li Q., Xie Q. G., Smith-Becker J., Navarre D. A., Kaloshian I.** (2006). Mi-1-mediated aphid resistance involves salicylic acid andmitogen-activated protein kinase signaling cascades. *Mol. Plant-Microbe Interact*. **19:**655–64

**Li Y., Zou J., Li M., Bilgin D. D., Vodkin L. O., Hartman G. L.** (2008). Soybean defense responses to the soybean aphid. New Phytologist. **179:** 185–195

**Liu S., Ruan W., Li J., Xu H., Wang J., Gao Y., Wang J.** (2008). Biological Control of Phytopathogenic Fungi by Fatty Acids. *Mycopathologia*. **166:**93–102

**Livak K.J., Schmittgen T.D.** (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25:** 402-408.

**Llorente F., Muskett P., Sánchez-Vallet A., López G., Ramos B., Sánchez-Rodríguez C., Jordá L., Parker J., Molina A.** (2008). Repression of the auxin response pathway increases Arabidopsis susceptibility to necrotrophic fungi. *Mol Plant*. **1(3):**496-509.

**López M. A., Bannenberg G. and Castresana C.** (2008). Controlling hormone signaling is a plant and pathogen challenge for growth and survival. *Current Opinion in Plant Biology* **11:** 420–427

**Lorenzo O., Chico J.M., Sanchez-Serrano J.J., and Solano R.** (2004). JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate regulated defense responses in Arabidopsis. *Plant Cell*. **16:** 1938–1950

**Lorenzo O., Piqueras R., Sánchez-Serrano J. J., Solano R.** (2003). ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. *Plant Cell.* **15(1):**165-78

**Luan S., Kudla J., Rodriguez-Concepcion M., Yalovsky S., Gruissem W.** (2002). Calmodulins and calcineurin B-like proteins: calcium sensors for specific signal response coupling in plants. *Plant Cell.* **14:** Suppl:S389-400

**Maffei M., Bossi S., Spiteller D., Mithofer A., Boland W.** (2004). Effects of feeding *Spodoptera littoralis* on lima bean leaves. I.Membrane potentials, intracellular calcium variations, oral secretions, and regurgitate components. *Plant Physiol*. **134:**1752–62

**Martineau B., McBride K.E., Mouck. C.M.** (1991) Regulation of metallocarboxypeptidase inhibitor gene expression in tomato. *Mol. Gen. Genet*. **228**: 281-286.

**Martinez de Ilarduya O., Xie Q. G. and Kaloshian I.** (2003). Aphid-induced defense responses in Mi-1-mediated compatible and incompatible tomato interactions. *Molecular Plant-Microbe Interactions* **16:** 699–708

**Mattiacci L.**, **Dicke M.**, **Posthumus M.A.**, (1995). Beta-glucosidase an elicitor of erbivore-induced plant odor that attracts host-searching parasitic wasps. *Proceedings of the National Academy of Sciences*, *USA* **92**: 2036-40.

**McGurl B., Orozco-Cárdenas M., Pearce G., Ryan C. A.** (1994). Overexpression of the prosystemin

**McGurl B., Pearce G., Orozco-Cárdenas M., Ryan C. A.** (1992). Structure, expression, and antisense

**McGurl B., Ryan C. A.** (1992). The organization of the prosystemin gene. *PlantMol Biol* **20:**405–409

**Meir S., Philosoph-Hadas S., Sundaresan S., Selvaraj K. S. V., Burd S., Ophir R., Kochanek B., Reid M. S., Jiang C.-Z., Lers A.** (2010). Microarray Analysis of the Abscission-Related Transcriptome in the Tomato Flower Abscission Zone in Response to Auxin Depletion. *Plant Physiol.* **154:** 1929–1956

**Meldau S., Wu J. Q., Baldwin I. T.** (2009). Silencing two herbivory-activated MAP kinases, SIPK and WIPK, does not increase *Nicotiana attenuata*'s susceptibility to herbivores in the glasshouse and in nature. *New Phytol.* **181:**161–73

**Melotto M., Underwood W. and He S. Y.** (2008). Role of stomata in plant innate immunity and foliar bacterial diseases. *Annual Review of Phytopathology* **46:** 101–122

**Miles P. W.** (1999). Aphid saliva. *Biological Reviews* **74:** 41–85

**Milligan S.B.**, **Bodeau J.**, **Yaghoobi J.**, **Kaloshian I.**, **Zabel P.**, **Williamson V.M.**, (1998).The root knot nematode resistance gene Mi from tomato is a member of the leucine zipper, nucleotide binding, leucine rich repeat family of plant genes. *Plant Cell* **10**:1307-1319.

**Minguez P., Götz S., Montaner D., Al-Shahrour F., Dopazo J.** (2009). SNOW, a web-based tool for the statistical analysis of protein-protein interaction networks. *Nucleic Acids Res.* 109-14.

**Mithofer A., Wanner G., Boland W.** (2005) Effects of feeding Spodoptera littoralis on lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. *Plant Physiology* **137**: 1160–1168.

**Morant A. V., Jørgensen K., Jørgensen C., Paquette S. M., Sánchez-Pèrez R., Møller B. L. and Bak S.** (2008). Glucosidases as detonators of plant chemical defense. *Phytochemistry* **69:** 1795–1813

**Müller R., de Vos M., Sun J. Y., Sønderby I. E., Halkier B. A., Wittstock U., Jander G.** (2010). Differential effects of indole and aliphatic glucosinolates on lepidopteran herbivores. *Journal of Chemical Ecology*. **36(8)**:905-13.

**Mur L. A. J., Kenton P., Atzorn R., Miersch O. and Wasternack C.** (2006). The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiology* **140:** 249–262

**Musser R.O., Cipollini D.F., Hum-Musser S.M., Williams S.A., Brown J.K., Felton G.W.** (2005) Evidence that the caterpillar salivary enzyme glucose oxidase provides herbivore offense in solanaceous plants. *Archives of Insect Biochemistry and Physiology* **58**: 128–137

**Naoumkina M. A., Zhao Q.,Gallego-giraldo L., Dai X., Zhao P., Dixon R. A.** (2010). Genome-wide analysis of phenylpropanoid defence pathways. *Molecular Plant Pathology*. **11(6):** 829–846

**Narváez-Vásquez J., Orozco-Cárdenas M. L., Ryan C. A.** (2007). Systemic wound signaling in tomato leaves is cooperatively regulated by multiple plant peptides. *Plant Mol Biol* **65:**711–718

**Narváez-Vásquez J., Pearce G., Ryan C. A.** (2005). The plant cell wall matrix harbors a precursor of defense signaling peptides. *Proc Natl Acad Sci USA* **102:**12974–12977

**Narváez-Vásquez J., Tu C. J., Park S. Y., Walling L.** (2008) Targeting and localization of wound-inducible leucine aminopeptidase A in tomato leaves. *Planta* **227**: 341–351

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

**Oerke E. C., Dehne W. H.** (2004). Safeguarding production—losses in major crops and the role of crop protection. *Crop Protection* **23**: 275–285

**Orozco-Cárdenas M. L., McGurl B., Ryan C. A.** (1993) Expression of an antisense prosystemin gene in tomato plants reduces resistance toward Manduca sexta larvae. *Proc Natl Acad Sci USA* **90:**8273–8276

**Orsini F., Cascone P., De Pascale S., Barbieri G., Corrado G., Rao R., Maggio A..** (2010). Systemin-dependent salinity tolerance in tomato: evidence of specific convergence of abiotic and biotic stress responses. *Physiol Plant*. **138(1):**10-21

**Panstruga R.** (2005). Serpentine plant MLO proteins as entry portals for powdery mildew fungi. *Biochem Soc Trans.* **33(Pt 2):**389-92.

**Park S. C., Kim Y. H., Jeong J. C., Kim C. Y., Lee H. S., Bang J. W., Kwak S. S.** (2011). Sweetpotato late embryogenesis abundant 14 (IbLEA14) gene influences lignification and increases osmotic- and salt stress-tolerance of transgenic calli. *Planta.* **233(3):**621-34

**Pearce G., Moura D. S., Stratmann J., Ryan C. A.** (2001). Production of multiple plant hormones from a single polyprotein precursor. *Nature* **411:**817–820

**Pearce G., Ryan C. A.** (2003). Systemic signaling in tomato plants for defense against herbivores- isolation and characterization of three novel defense-signaling glycopeptide hormones coded in a single precursor gene. J Biol Chem **278:**30044–30050

**Pedley K. F., Martin G. B.** (2004). Identification of MAPKs and their possible MAPK kinase activators involved in the Pto-mediated defense response of tomato. *J Biol Chem*. **279(47):**49229-35

**Pokalsky A.R., Hiatt W.R., Ridge N., Rasmussen R., Houck C.M., Shewmaker C.K.** (1989). Structure and expression of elongation factor 1 alpha in tomato. *Nucleic Acids Res* **17**: 4661-4673.

**Price D. R., Tibbles K., Shigenobu S., Smertenko A., Russell C. W., Douglas A. E., Fitches E., Gatehouse A. M., Gatehouse J. A.** (2010). Sugar transporters of the major facilitator superfamily in aphids; from gene prediction to functional characterization. *Insect Mol Biol.* **2:**97-112

proteinase-inhibitor synthesis. *Proc Natl Acad Sci USA* **91:**9799–9802

**Rath A., Davison A. R., Deber C. M.** (2005). The structure of 'unstructured' regions in peptides and proteins: role of the polyproline II helix in protein folding and recognition. *Biopolymers* **80:** 179–185

**Rocco M., Corrado G., Arena S., D'Ambrosio C., Tortiglione C., Sellaroli S., Marra M., Rao A., Scaloni A.** (2008). The expression of tomato prosystemin gene in tobacco plants highly affects host proteomic repertoire. *Journal of Proteomics* **71:** 176-185

**Rodriguez-Saona C. R., Musser R. O., Vogel H., Hum-Musser S. M., Thaler J. S.** (2010). Molecular, biochemical, and organismal analyses of tomato plants simultaneously attacked by herbivores from two feeding guilds. *J Chem Ecol.* **36(10):**1043-57

**Romero-Puertas M.C., Campostrini N., Mattè A., Righetti P.G., Perazzolli M., Zolla L., Roepstorff P., Delledonne M.** (2008). Proteomic analysis of S-nitrosylated proteins in Arabidopsis thaliana undergoing hypersensitive response. *Proteomics* **8(7):**1459-69

**Rossi M.**, **Goggin F.L.**, **Milligan S.B.**, **Kaloshian I.**, **Ullman D.E.**, **Williamson V.M.**, (1998). The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. *Proceedings of the National Academy of Sciences, USA* **95**: 9750-9754.

**Ruiz-Medrano R., Xoconostle-Cazares B., Lucas W. J.** (2001). The phloem as a conduit for inter-organ communication. *Current Opinion in Plant Biology* **4:** 202–209.

**Ryan C. A.** (1985). Wound-induced Proteinase Inhibitors from Tomato Leaves. *Journal of biological chemistry***. 260:** 6555-6560

**Ryan C. A.** (2000). The systemin signalling pathway: differential activation of plant defensive genes. *Biochimica et Biophysica Acta-Protein Structure et Molecular Enzymologia* **1477**:112-121.

**Ryan C. A., Pearce G.** (1998). Systemin: a polypeptide signal for plant defensive genes. *Annu Rev Cell Dev Biol.* **14:**1-17

**Sagi M., Davydov O., Orazova S., Yesbergenova Z., Ophir R.** (2004). Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in Lycopersicon esculentum. *Plant Cell* **16:**616–28

**Samach A., Hareven D., Gutfinger T., Ken-Dror S., Lifschitz E.** (1991). Biosynthetic threonine deaminase gene of tomato: isolation, structure, and upregulation in floral organs. *Proc Natl Acad Sci U S A*. 88(7):2678-82.

**Sambrook J.**, **Fritsch E.F.**, **Maniatis T**. (1989). Molecular cloning: A laboratory manual 2nd edition. *Cold Spring Harbor Laboratory Press*.

**Schaller A.** (2008). Induced plant resistance to herbivory. *Springer*. ISBN 1402081812, 9781402081811

**Scheer J. M., Pearce G., Ryan C. A.** (2003). Generation of systemin signaling in tobacco by transformation

**Schilmiller A. L., Howe G. A.** (2005). Systemic signaling in the wound response. *Curr Opin Plant Biol.* **8(4):**369-77.

**Schmelz E. A., Alborn A. T., Engelberth J., Tumlinson J. H.** (2003). Nitrogen Deficiency Increases Volicitin-Induced Volatile Emission, Jasmonic Acid Accumulation, and Ethylene Sensitivity in Maize. *Plant Physiology.* **133:** 295–306

**Schmelz E. A., LeClere S., Carroll M. J., Alborn H. T., Teal P. E.** (2007). Cowpea chloroplastic ATP synthase is the source of multiple plant defense elicitors during insect herbivory. *Plant Physiol.* **144:**793–805

**Schmelz E. A., Engelberth J., Alborn A. T., Tumlinson J. H., Teal P. E. A.** (2009). Phytohormone-based activity mapping of insect herbivore-produced elicitors. *PNAS.* **106(2):** 653-657

**Schweighofer A., Hirt H., Meskiene I.** (2004). Plant PP2C phosphatases: emerging functions in stress signalling. *TRENDS in Plant Science.* **9(5):** 236-247

**Shewmaker C.K., Ridge N.P., Pokalsky A.R., Rose R.E., Hiatt W.R.** (1990) Nucleotide sequence of an EF-1 alpha genomic clone from tomato. *Nucleic Acids Res.* **14**, 4276

**Singh K., Foley R. C., Oñate-Sánchez L.** (2002). Transcription factors in plant defense and stress responses. *Curr Opin Plant Biol.* **5(5):**430-6

**Sivaguru M., Ezaki B., He Z-H., Tong H., Osawa H., Baluska F., Volkmann D., Matsumoto H.** (2003) Aluminum induced gene expression and protein localization of a cell wall-associated receptor protein kinase in *Arabidopsis thaliana. Plant Physiol* **132:** 2256–2266

**Smith C. M., Boyko E. V.** (2006). The molecular bases of plant resistance and defense responses to aphid feeding: current status. *Entomologia Experimentalis et Applicata* **122:** 1–16

**Smith C. M., Liu X., Wang L. J., Liu X., Chen M. S., Starkey S., Bai J.** (2010). Aphid feeding activates expression of a transcriptome of oxylipin-based defense signals in wheat involved in resistance to herbivory. *J Chem Ecol*. **36(3):**260-76

**Soltis P. S., Soltis D. E., Chase M. W.** (1999). Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature*. **402:** 402–404

**Spoel SH, Johnson JS, Dong X.** (2007). Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. *Proc Natl Acad Sci U S A.* **104(47):**18842-7

**Staswick P. E., Tiryaki I.** (2004). The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. *Plant Cell* **16:**2117–2127

**Sun J., Jiang H., Li C.** (2011). Systemin/Jasmonate-Mediated Systemic Defense Signaling in Tomato. *Molecular Plant* **4(4):** 607-615

**Taji T., Seki M., Yamaguchi-Shinozaki K., Kamada H., Giraudat J., Shinozaki K.** (1999). Mapping of 25 Drought-Inducible Genes, *RD* and *ERD,* in *Arabidopsis thaliana. Plant Cell Physiol.* **40(1):** 119-123

**Tamba Y., Ohba S., Kubota M., Yoshioka H., Yoshioka H., Yamazaki M.** (2007). Single GUV method reveals interaction of tea catechin (-)-epigallocatechin gallate with lipid membranes. *Biophys J.* **92(9):**3178-94

**Taylor C. B.** (1997). Comprehending cosuppression. *The Plant Cell.* **9:** 1245-1249

**Tewksbury J. J., Levey D. J., Huizinga M., Haak D. C., Traveset A.** (2008). Costs and benefits of capsaicin-mediated control of gut retention in dispersers of wild chilies. *Ecology.* **89(1):**107-17

**Thines B., Katsir L., Melotto M. (2007).** JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. *Nature* **448:**661–665

**Tortiglione C., Fogliano V., Ferracane R., Fanti P., Pennacchio F., Monti L.M., Rao R.** (2003). An insect peptide engineered into the tomato prosystemin gene is released in transgenic tabacco plants and exerts biological activity. *Plant Molecular Biology* **53:** 891-902

**Turlings T. C. J., McCall P. J., Alborn H. T. and Tumlinson J. H.** (1993). An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *Journal of Chemical Ecology* **19:** 411–425

**Ueda K., Kosako H., Fukui Y., Hattori S.** (2004). Proteomic identification of Bcl2-associated athanogene 2 as a novel MAPK-activated protein kinase 2 substrate. *J Biol Chem.* **279(40):**41815-21.

**Van der Hoeven R., Ronning C., Giovannoni J., Martin G., Tanksley S.** (2002). Deductions about the number, organization, and evolution of genes in the tomato genome based on analysis of a large expressed sequence tag collection and selective genomic sequencing. *Plant Cell.* **14:** 1441–1456

**van Roekel, J., Damm, B., Melchers, L., e Hoekema, A.** (1993). Factors influencing transformation frequency of tomato (Lycopersicon esculentum). Plant Cell Reports **12:** 644-647

**Vandeborre G., Smagghe G., Van Damme E. J. M.** (2011). Plant lectins as defence proteins against phytophagous insects. *Phytochemistry* **72:** 1538-1550

**Verheggen F. J., Arnaud L., Bartram S., Gohy M., Haubruge E.** (2008). Aphid and Plant Volatiles Induce Oviposition in an Aphidophagous Hoverfly. *Journal of Chemical Ecology.* **34:**301–307

**Vincent J.B., Crowder M.W., Averill B.A.** (1992) Hydrolysis of phosphate monoesters: a biological problem with multiple chemical solutions. *Trends Biochem. Sci.* **17:** 105–110.

**von Dahl CC, Winz RA, Halitschke R, Kühnemann F, Gase K, Baldwin IT.** (2007). Tuning the herbivore-induced ethylene burst: the role of transcript accumulation and ethylene perception in *Nicotiana attenuata*. *Plant Journal* **51(2):**293-307

**Walling L.L.**, (2000). The myriad plant responses to herbivores. *Journal of Plant Growth Regulation* **19**: 195-216.

**Walling L.L.**, (2008). Avoiding effective defenses: Strategies employed by phloem feeding insects. *Plant Physiology* **146:** 859–866

**Walling L.L.**, (2009). Adaptive Defense Responses to Pathogens and Insects. *Advances in Botanical Research.* **51**: 552-612

**Walters D. R.** (2003). Polyamines and plant diseases. *Phytochemistry* **64:**97-107

**Wander J. G. N., Bouwmeester H. J.** (1998). Effects of nitrogen fertilization on dill (*Anethum graveolens L.*) seed and carrone production. *Ind Crops Prod* **7:** 211–216

**Werner R., Guitton M. C., Mühlbach H. P.** (1993). Nucleotide sequence of a cathepsin D inhibitor protein from tomato. *Plant Physiol.* **103(4):**1473

**Whenham R. J., Fraser R. S.** (1982). Does tobacco mosaic virus RNA contain cytokinins? *Virology* **118(1):** 263-6

**Williamson V.M.**, **Ho J-H.**, **Wu F.F.**, **Miller N.**, **Kaloshian I.**, (1994). A PCR-based marker tightly linked to the nematode resistance gene, *Mi*, in tomato. *Theoretical and Applied Genetics* **87**: 757-763.

**Wimalasekera R., Tebartz F., Scherer G. F.** (2011). Polyamines, polyamine oxidases and nitric oxide in development, abiotic and biotic stresses. *Plant Sci.* **181(5):**593-603.

with the tomato systemin receptor kinase gene. *Proc Natl Acad Sci USA* **100:**10114–10117

**Wong J.H., Ng T.B., Cheung R.C., Ye X.J., Wang H.X., Lam S.K., Lin P., Chan Y.S., Fang E. F., Ngai P. H., Xia L. X., Ye X. Y., Jiang Y., Liu F**. (2008). Proteins with antifungal properties and other medicinal applications from plants and mushrooms. *Appl. Microbiol. Biotechnol.* **87**: 1221-1235

**Wu and Baldwin I. T**. (2009). Herbivory-induced signalling in plants: perception and action. *Plant, Cell and Environment.* **32(9):**1161-74

**Wu and Baldwin I. T**. (2010) New insights into plant responses to the attack from insect herbivores. *Annual Reviews of Genetics.* **44**: 1-24

**Wu J., Hettenhausen C., Meldau S., Baldwin I. T.** (2007). Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of *Nicotiana attenuata*. *Plant Cell* 19:1096–122

**Xie D. X., Feys B. F., James S., Nieto-Rostro M., Turner J. G.** (1998). COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science **280:**1091–1094

**Yamaguchi Y., Pearce G., Ryan C. A.** (2006). The cell surface leucine-rich repeat receptor for AtPep1, an endogenous peptide elicitor in *Arabidopsis*, is functional in transgenic tobacco cells. *Proc Natl Acad Sci USA* **103:**10104-10109

**Yao L., Zhou Q., Pei B., Li Y.** (2011). Hydrogen peroxide modulates the dynamic microtubule cytoskeleton during the defence responses to *Verticillium dahliae* toxins in *Arabidopsis. Plant, Cell and Environment*. **34**: 1586–159

**Zarate S. I., Kempema L. A., Walling L. L.** (2007). Silverleaf Whitefly Induces Salicylic Acid Defenses and Suppresses Effectual Jasmonic Acid Defenses. *Plant Physiology* **143:** 866–875

**Zhang C., Shi1 H., Chen L., Wang X., Lü B., Zhang S., Liang Y, Liu R., Qian J., Sun W., You Z., Dong H.** (2011). Harpin-induced expression and transgenic overexpression of the phloem protein gene AtPP2-A1 in *Arabidopsis* repress phloem feeding of the green peach aphid *Myzus persicae. BMC Plant Biology*. **11:** 1-19

**Zhang Z., Wang M., Li Z., Li Q., He Z.** (2008). Arabidopsis GH3.5 regulates salicylic acid-dependent and both NPR1-dependent and independent defense responses. *Plant Signaling & Behavior* **3(8):** 537-542

**Zhao H., Yang H.** (2008). Exogenous polyamines alleviate the lipid peroxidation induced by cadmium chloride stress in *Malus hupehensis Rehd. Sci Hortic*. **116:**442–7

**Zhu S. Y., Yu X. C., Wang X. J., Zhao R., Li Y.** (2007). Two calcium-dependent protein kinases, CPK4 and CPK11, regulate abscisic acid signal transduction in Arabidopsis. *Plant Cell* **19:**3019–36

**Zhu-Salzman K., Bi J.-L., Liu T.-X.** (2005). Molecular strategies of plant defense and insect counter-defense. Insect Sci **12:** 3–15

**Zhu-Salzman K., Luthe D. S., Felton G. W.** (2008). Arthropod-Inducible Proteins: Broad Spectrum Defenses against Multiple Herbivores. *Plant Physiology* **146:** 852–858

# 6. APPENDIX

## Table A1 Differentially espressed sequences by ProSys over-expression.

| Codes | ProbeName | FC | Reg | Description | GOs |
|---|---|---|---|---|---|
| AF198390 | A_96_P263412 | 222,162 | up | cysteine protease inhibitor | F:hydrolase activity; F:enzyme regulator activity |
| AF083253 | A_96_P016051 | 211,722 | up | cysteine proteinase inhibitor | F:hydrolase activity; F:enzyme regulator activity |
| AJ289776 | A_96_P264952 | 148,352 | up | kunitz-type protease inhibitor precursor | C:vacuole; F:enzyme regulator activity; P:response to stress; F:hydrolase activity |
| X73986 | A_96_P073229 | 119,806 | up | kunitz-type protease inhibitor precursor | C:vacuole; F:enzyme regulator activity; P:response to stress; F:hydrolase activity |
| AI488671 | A_96_P132022 | 94,568 | up | proteinase inhibitor i | F:enzyme regulator activity |
| K03291 | A_96_P195459 | 92,125 | up | proteinase inhibitor ii | C:extracellular region; F:enzyme regulator activity; F:hydrolase activity |
| K03291 | A_96_P163535 | 89,416 | up | proteinase inhibitor ii | C:extracellular region; F:enzyme regulator activity; F:hydrolase activity |
| K03291 | A_96_P179565 | 88,317 | up | proteinase inhibitor ii | C:extracellular region; F:enzyme regulator activity; F:hydrolase activity |
| U50152 | A_96_P000336 | 82,201 | up | leucine aminopeptidase | C:vacuole; F:binding; F:hydrolase activity; P:biological_process; P:response to stress; C:plastid; P:protein metabolic process; P:catabolic process |
| K03291 | A_96_P090049 | 79,951 | up | proteinase inhibitor ii | C:extracellular region; F:enzyme regulator activity; F:hydrolase activity |
| AK319505 | A_96_P192049 | 79,947 | up | leucine aminopeptidase | C:vacuole; F:binding; F:hydrolase activity; P:biological_process; P:response to stress; C:plastid; P:protein metabolic process; P:catabolic process |
| K03291 | A_96_P193109 | 74,604 | up | proteinase inhibitor ii | C:extracellular region; F:enzyme regulator activity; F:hydrolase activity |
| AY534531 | A_96_P173409 | 71,321 | up | alcohol acyl transferase | F:transferase activity; P:biosynthetic process |
| BI928789 | A_96_P204319 | 69,723 | up | prosystemin | F:receptor binding; C:cytoplasm; P:signal transduction |
| M84801 | A_96_P000991 | 65,441 | up | prosystemin | F:receptor binding; C:cytoplasm; P:signal transduction |
| U50152 | A_96_P165811 | 61,812 | up | leucine aminopeptidase | C:vacuole; F:binding; F:hydrolase activity; P:biological_process; P:response to stress; C:plastid; P:protein metabolic process; P:catabolic process |
| U50152 | A_96_P165006 | 61,613 | up | leucine aminopeptidase | C:vacuole; F:binding; F:hydrolase activity; P:biological_process; P:response to stress; C:plastid; P:protein metabolic process; P:catabolic process |
| M84801 | A_96_P131637 | 52,550 | up | prosystemin | F:receptor binding; C:cytoplasm; P:signal transduction |
| AW738222 | A_96_P011621 | 49,618 | up | trypsin and protease inhibitor family protein | F:enzyme regulator activity; F:hydrolase activity |
| DV935725 | A_96_P247602 | 49,280 | up | | zinc binding dehydrogenase |

| | | | | | |
|---|---|---|---|---|---|
| GO372295 | A_96_P192054 | 48,347 | up | metallocarboxypeptidase inhibitor precursor | F:enzyme regulator activity |
| M84801 | A_96_P174964 | 46,358 | up | prosystemin | F:receptor binding; C:cytoplasm; P:signal transduction |
| BI933034 | A_96_P011231 | 38,990 | up | kunitz trypsin inhibitor | F:hydrolase activity; F:enzyme regulator activity |
| AI487256 | A_96_P131422 | 28,880 | up | endoribonuclease l-psp family protein | P:biological_process; C:vacuole; C:thylakoid; C:mitochondrion; C:plastid; F:nuclease activity; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process |
| BT013158 | A_96_P014141 | 24,887 | up | polyphenol oxidase | F:binding; F:catalytic activity; C:plastid; C:thylakoid; P:metabolic process; P:cellular amino acid and derivative metabolic process |
| K03290 | A_96_P093059 | 24,006 | up | proteinase inhibitor i | C:extracellular region; F:enzyme regulator activity; P:response to external stimulus; P:response to stress; F:hydrolase activity |
| TA35655_4081 | A_96_P086739 | 23,065 | up | polyphenol oxidase | F:binding; F:catalytic activity; C:plastid; C:thylakoid; P:metabolic process; P:cellular amino acid and derivative metabolic process |
| K03290 | A_96_P074134 | 22,997 | up | proteinase inhibitor i | C:extracellular region; F:enzyme regulator activity; P:response to external stimulus; P:response to stress; F:hydrolase activity |
| K03290 | A_96_P193924 | 22,594 | up | proteinase inhibitor i | C:extracellular region; F:enzyme regulator activity; P:response to external stimulus; P:response to stress; F:hydrolase activity |
| GO372786 | A_96_P011246 | 22,523 | up | endoribonuclease l-psp family protein | P:biological_process; C:vacuole; C:thylakoid; C:mitochondrion; C:plastid; F:nuclease activity; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process |
| BG627206 | A_96_P082889 | 22,122 | up | ---NA--- | ---NA--- |
| BI925947 | A_96_P202714 | 20,102 | up | polyphenol oxidase | F:binding; F:catalytic activity; C:plastid; C:thylakoid; P:metabolic process; P:cellular amino acid and derivative metabolic process; P:cellular process; P:biosynthetic process; P:secondary metabolic process |
| TA35793_4081 | A_96_P087054 | 19,125 | up | polyphenol oxidase | F:binding; F:catalytic activity; C:plastid; C:thylakoid; P:metabolic process; P:cellular amino acid and derivative metabolic process; P:cellular process; P:biosynthetic process; P:secondary metabolic process |
| M61914 | A_96_P205969 | 17,877 | up | threonine deaminase | P:cellular amino acid and derivative metabolic process; F:binding; P:response to stress; P:biosynthetic process; F:catalytic activity; C:plastid |
| AK247410 | A_96_P071734 | 17,816 | up | polyphenol oxidase | F:binding; F:catalytic activity; C:plastid; C:thylakoid; P:metabolic process; P:cellular amino acid and derivative metabolic process; P:cellular process; P:biosynthetic process; P:secondary metabolic process |
| AK321112 | A_96_P011291 | 17,497 | up | arginase | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:catabolic process; C:mitochondrion; P:response to stress; P:response to biotic stimulus; F:binding; F:hydrolase activity; C:plastid |
| AK247410 | A_96_P086869 | 17,056 | up | polyphenol oxidase | F:binding; F:catalytic activity; C:plastid; C:thylakoid; P:metabolic process; P:cellular amino acid and derivative metabolic process; P:cellular process; P:biosynthetic process; P:secondary metabolic process |
| M61914 | A_96_P131932 | 16,777 | up | threonine deaminase | P:cellular amino acid and derivative metabolic process; F:binding; P:response to stress; P:biosynthetic process; F:catalytic activity; C:plastid |
| M61914 | A_96_P129792 | 16,604 | up | threonine deaminase | P:cellular amino acid and derivative metabolic process; F:binding; P:response to stress; P:biosynthetic process; F:catalytic activity; C:plastid |

| | | | | | |
|---|---|---|---|---|---|
| M61914 | A_96_P171334 | 16,437 | up | threonine deaminase | P:cellular amino acid and derivative metabolic process; F:binding; P:response to stress; P:biosynthetic process; F:catalytic activity; C:plastid |
| M61914 | A_96_P165486 | 15,834 | up | threonine deaminase | P:cellular amino acid and derivative metabolic process; F:binding; P:response to stress; P:biosynthetic process; F:catalytic activity; C:plastid |
| ES897217 | A_96_P171804 | 15,529 | up | kunitz-type protease inhibitor precursor | C:vacuole; F:enzyme regulator activity; P:response to stress; F:hydrolase activity |
| M61914 | A_96_P171829 | 14,584 | up | threonine deaminase | P:cellular amino acid and derivative metabolic process; F:binding; P:response to stress; P:biosynthetic process; F:catalytic activity; C:plastid |
| ES897217 | A_96_P129487 | 14,225 | up | kunitz-type protease inhibitor precursor | C:vacuole; F:enzyme regulator activity; P:response to stress; F:hydrolase activity |
| M61914 | A_96_P034156 | 13,875 | up | threonine deaminase | P:cellular amino acid and derivative metabolic process; F:binding; P:response to stress; P:biosynthetic process; F:catalytic activity; C:plastid |
| TA35655_4081 | A_96_P160021 | 13,846 | up | polyphenol oxidase | F:binding; F:catalytic activity; C:plastid; C:thylakoid; P:metabolic process; P:cellular amino acid and derivative metabolic process |
| U50152 | A_96_P165596 | 13,628 | up | leucine aminopeptidase | C:vacuole; F:binding; F:hydrolase activity; P:biological_process; P:response to stress; C:plastid; P:protein metabolic process; P:catabolic process |
| AY656837 | A_96_P017316 | 13,575 | up | arginase | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:catabolic process; C:mitochondrion; P:response to stress; P:response to biotic stimulus; F:binding; F:hydrolase activity; C:plastid |
| AK323732 | A_96_P010533 | 13,567 | up | stem 28 kda glycoprotein | C:cytoplasm; F:hydrolase activity |
| BI931087 | A_96_P040851 | 12,996 | up | myb-related transcription factor | P:response to endogenous stimulus; F:DNA binding; P:transcription; P:biological_process; C:nucleus; P:response to stress; P:response to abiotic stimulus |
| AK320125 | A_96_P071899 | 12,940 | up | anthranilate n-hydroxycinnamoyl benzoyltransferase | F:transferase activity |
| AK247121 | A_96_P032621 | 12,794 | up | unnamed protein product [Vitis vinifera] | |
| ES897217 | A_96_P165661 | 11,614 | up | kunitz-type protease inhibitor precursor | C:vacuole; F:enzyme regulator activity; P:response to stress; F:hydrolase activity |
| AK320918 | A_96_P197769 | 10,797 | up | protein | P:metabolic process; F:catalytic activity; F:binding; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| BI928680 | A_96_P204254 | 9,855 | up | leucine aminopeptidase | C:vacuole; F:binding; F:hydrolase activity; P:biological_process; P:response to stress; C:plastid; P:protein metabolic process; P:catabolic process |
| AI485880 | A_96_P073839 | 9,769 | up | wrky transcription factor 1 | F:protein binding; P:response to stress; P:transcription |
| TA38526_4081 | A_96_P093601 | 9,551 | up | subtilisin-like protease | P:biological_process; P:protein metabolic process; P:catabolic process; F:protein binding; F:hydrolase activity |
| AW648744 | A_96_P054411 | 9,537 | up | iaa-amino acid hydrolase ilr1 | F:protein binding; F:hydrolase activity; P:cellular amino acid and derivative metabolic process |

| | | | | | |
|---|---|---|---|---|---|
| BI931371 | A_96_P074099 | 9,073 | up | polyphenol oxidase | F:binding; F:catalytic activity; C:plastid; C:thylakoid; P:metabolic process; P:cellular amino acid and derivative metabolic process |
| AK328079 | A_96_P011176 | 8,828 | up | peptidase m20 m25 m40 family protein | F:hydrolase activity; P:protein metabolic process; P:catabolic process; F:protein binding |
| AJ271093 | A_96_P000051 | 8,760 | up | allene oxide synthase | F:catalytic activity; F:binding; F:molecular_function |
| BI924537 | A_96_P201884 | 8,633 | up | purple acid phosphatase | C:cell; C:cytoplasm; F:binding; F:hydrolase activity; F:protein binding; P:protein modification process; P:metabolic process; P:cellular process |
| AI485880 | A_96_P086299 | 8,627 | up | wrky transcription factor 1 | F:protein binding; P:response to stress; P:transcription |
| AK328079 | A_96_P092084 | 8,557 | up | peptidase m20 m25 m40 family protein | F:hydrolase activity; P:protein metabolic process; P:catabolic process; F:protein binding |
| TA41294_4081 | A_96_P098414 | 8,106 | up | protein | F:transferase activity |
| AK328079 | A_96_P150451 | 7,835 | up | peptidase m20 m25 m40 family protein | F:hydrolase activity; P:protein metabolic process; P:catabolic process; F:protein binding |
| AF146690 | A_96_P012966 | 7,682 | up | pto-responsive gene 1 protein | |
| AW944751 | A_96_P010901 | 7,639 | up | proteinase inhibitor i | C:extracellular region; F:enzyme regulator activity; P:response to external stimulus; P:response to stress; F:hydrolase activity |
| AK329477 | A_96_P042771 | 7,250 | up | pr-10 type pathogenesis-related protein | P:response to biotic stimulus; P:response to stress |
| AK321887 | A_96_P055516 | 7,171 | up | extensin-like protein | P:transport; C:cell; F:lipid binding |
| EG553806 | A_96_P054926 | 7,127 | up | protein | F:molecular_function; P:biological_process; C:cellular_component |
| AJ271093 | A_96_P106059 | 6,928 | up | allene oxide synthase | F:catalytic activity; F:binding; F:molecular_function |
| AW429114 | A_96_P155641 | 6,485 | up | ac091627_9amidase family protein | C:cytoplasm; F:catalytic activity |
| AI485880 | A_96_P131922 | 6,484 | up | wrky transcription factor 1 | F:protein binding; P:response to stress; P:transcription |
| AF146690 | A_96_P117212 | 6,467 | up | pto-responsive gene 1 protein | |
| AK327616 | A_96_P129917 | 6,160 | up | protein | F:binding; F:hydrolase activity; P:metabolic process; P:cellular process; P:protein modification process; C:cell |
| BI931662 | A_96_P021601 | 6,098 | up | auxin efflux carrier family protein | P:transport; C:cell; C:membrane; F:transporter activity; P:cellular process |
| BI927096 | A_96_P053681 | 5,973 | up | protein | F:transferase activity |
| BI924994 | A_96_P202114 | 5,879 | up | protein | F:hydrolase activity; C:cytoplasm; F:catalytic activity; P:cellular amino acid and derivative metabolic process; P:metabolic process; P:cellular process; P:catabolic process |
| BI206321 | A_96_P023961 | 5,791 | up | calcium-dependent protein kinase | P:biosynthetic process; P:cellular process; F:kinase activity; P:protein modification process; F:protein binding; F:binding; F:nucleotide binding; P:cellular amino acid and derivative metabolic process |

| | | | | | |
|---|---|---|---|---|---|
| AK320918 | A_96_P042931 | 5,761 | up | protein | P:metabolic process; F:catalytic activity; F:binding; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| AI486264 | A_96_P257717 | 5,754 | up | anthocyanin acyltransferase | F:transferase activity |
| BI206321 | A_96_P182644 | 5,625 | up | calcium-dependent protein kinase | P:biosynthetic process; P:cellular process; F:kinase activity; P:protein modification process; F:protein binding; F:binding; F:nucleotide binding; P:cellular amino acid and derivative metabolic process |
| AK322858 | A_96_P100214 | 5,618 | up | 3-ketoacyl- thiolase | P:cellular component organization; P:cellular process; C:peroxisome; C:mitochondrion; C:nucleolus; F:transferase activity; P:response to external stimulus; P:response to stress; P:biosynthetic process; P:lipid metabolic process; P:catabolic process; C:plastid; C:plasma membrane; P:cellular amino acid and derivative metabolic process; P:metabolic process |
| BG129343 | A_96_P262272 | 5,550 | up | anthocyanin acyltransferase | F:transferase activity; P:biological_process |
| AI486811 | A_96_P096269 | 5,494 | up | polyphenol oxidase | F:binding; F:catalytic activity; C:plastid; C:thylakoid; P:metabolic process; P:cellular amino acid and derivative metabolic process; P:cellular process; P:biosynthetic process; P:secondary metabolic process |
| AK322858 | A_96_P077334 | 5,421 | up | 3-ketoacyl- thiolase | P:cellular component organization; P:cellular process; C:peroxisome; C:mitochondrion; C:nucleolus; F:transferase activity; P:response to external stimulus; P:response to stress; P:biosynthetic process; P:lipid metabolic process; P:catabolic process; C:plastid; C:plasma membrane; P:cellular amino acid and derivative metabolic process; P:metabolic process |
| AY840091 | A_96_P001996 | 5,369 | up | (-)-a-terpineol synthase | P:biosynthetic process; P:cellular process; P:lipid metabolic process; P:secondary metabolic process; F:binding; F:catalytic activity |
| BI207729 | A_96_P033061 | 5,360 | up | jasmonate zim-domain protein 1 | P:response to endogenous stimulus; P:signal transduction; P:response to stress; P:response to biotic stimulus |
| TA37286_4081 | A_96_P091149 | 5,348 | up | wrky transcription factor 1 | F:DNA binding; P:transcription; F:transcription factor activity; C:nucleoplasm |
| AW035871 | A_96_P146991 | 5,338 | up | lactoylglutathione lyase | F:catalytic activity |
| BG626001 | A_96_P191404 | 5,337 | up | subtilisin-like protease | P:protein metabolic process; P:catabolic process; F:hydrolase activity |
| AK325215 | A_96_P018261 | 5,310 | up | beta-alanine-pyruvate aminotransferase | F:transferase activity; F:binding; P:response to extracellular stimulus; P:cell communication; C:mitochondrion; P:cellular amino acid and derivative metabolic process |
| AK325215 | A_96_P055841 | 5,289 | up | beta-alanine-pyruvate aminotransferase | F:transferase activity; F:binding; P:response to extracellular stimulus; P:cell communication; C:mitochondrion; P:cellular amino acid and derivative metabolic process |
| TA54114_4081 | A_96_P116022 | 5,269 | up | MYB transcription factor | P:response to stress; P:response to abiotic stimulus; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process; P:transcription; P:response to external stimulus; P:biological_process; P:metabolic process; P:response to endogenous stimulus; F:protein binding; F:transcription factor activity; C:nucleoplasm |
| AK323178 | A_96_P130852 | 5,268 | up | protein | C:vacuole; C:cell wall; F:hydrolase activity; P:catabolic process; P:lipid metabolic process |
| TA43917_4081 | A_96_P141222 | 5,236 | up | xanthine dehydrogenase | P:metabolic process; P:cellular process; F:catalytic activity; F:molecular_function; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; P:catabolic process; F:binding; P:response to stress; F:nucleotide binding |
| TA48362_4081 | A_96_P106494 | 5,193 | up | allene oxide synthase | F:catalytic activity; F:binding; F:molecular_function |

| | | | | | |
|---|---|---|---|---|---|
| AK327683 | A_96_P248562 | 4,978 | up | jasmonate zim-domain protein 1 | P:response to endogenous stimulus; P:multicellular organismal development; P:response to stress |
| AK323178 | A_96_P097679 | 4,950 | up | protein | C:vacuole; C:cell wall; F:hydrolase activity; P:catabolic process; P:lipid metabolic process |
| AK319704 | A_96_P094299 | 4,949 | up | anthocyanin acyltransferase | F:transferase activity; P:biological_process |
| TA42014_4081 | A_96_P099419 | 4,945 | up | 7-transmembrane G-protein-coupled receptor | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:response to endogenous stimulus; F:receptor activity; P:signal transduction |
| DB685882 | A_96_P012376 | 4,942 | up | ---NA--- | |
| AK323178 | A_96_P132817 | 4,935 | up | protein | C:vacuole; C:cell wall; F:hydrolase activity; P:catabolic process; P:lipid metabolic process |
| ES894405 | A_96_P191629 | 4,738 | up | s-adenosyl-l-methionine:benzoic acid salicylic acid carboxyl methyltransferase | F:transferase activity |
| TA37091_4081 | A_96_P090714 | 4,720 | up | spermidine synthase | C:cytoplasm; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:protein binding; F:transferase activity; P:cellular process; P:secondary metabolic process |
| AK319704 | A_96_P040616 | 4,634 | up | anthocyanin acyltransferase | F:transferase activity; P:biological_process |
| AK327683 | A_96_P092699 | 4,627 | up | jasmonate zim-domain protein 1 | P:response to endogenous stimulus; P:multicellular organismal development; P:response to stress |
| AK327683 | A_96_P043846 | 4,623 | up | jasmonate zim-domain protein 1 | P:response to endogenous stimulus; P:multicellular organismal development; P:response to stress |
| AK323178 | A_96_P074399 | 4,557 | up | protein | C:vacuole; C:cell wall; F:hydrolase activity; P:catabolic process; P:lipid metabolic process |
| BP878428 | A_96_P210104 | 4,502 | up | subtilisin-like protease | F:hydrolase activity |
| AW626331 | A_96_P160891 | 4,498 | up | polyphenol oxidase | F:binding; F:catalytic activity; C:plastid; C:thylakoid; P:metabolic process; P:cellular amino acid and derivative metabolic process; P:cellular process; P:biosynthetic process; P:secondary metabolic process |
| DB685882 | A_96_P150576 | 4,486 | up | ---NA--- | |
| AK324059 | A_96_P233829 | 4,458 | up | spermidine synthase | C:cytoplasm; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:protein binding; F:transferase activity; P:cellular process; P:secondary metabolic process |
| BI933305 | A_96_P206079 | 4,395 | up | mate efflux family protein | P:response to biotic stimulus; C:vacuole; C:plasma membrane; P:transport; P:cellular process; F:transporter activity |
| BI206363 | A_96_P197229 | 4,341 | up | mate efflux family protein | P:response to biotic stimulus; C:vacuole; F:transporter activity; C:plasma membrane; P:transport; P:cellular process |
| AI898504 | A_96_P141022 | 4,336 | up | ac007354_12 gb | C:cell; C:cytoplasm |
| AK324059 | A_96_P090719 | 4,330 | up | spermidine synthase | C:cytoplasm; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:protein binding; F:transferase activity; P:cellular process; P:secondary metabolic process |
| AK324059 | A_96_P244935 | 4,309 | up | spermidine synthase | C:cytoplasm; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:protein binding; F:transferase activity; P:cellular process; P:secondary metabolic process |
| AK324059 | A_96_P199929 | 4,287 | up | spermidine synthase | C:cytoplasm; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:protein binding; F:transferase activity; P:cellular process; P:secondary metabolic process |

| | | | | | |
|---|---|---|---|---|---|
| AK325215 | A_96_P055491 | 4,274 | up | beta-alanine-pyruvate aminotransferase | F:transferase activity; F:binding; P:response to extracellular stimulus; P:cell communication; C:mitochondrion; P:cellular amino acid and derivative metabolic process |
| BG132352 | A_96_P189034 | 4,244 | up | spermidine synthase | C:cytoplasm; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:protein binding; F:transferase activity |
| BI924804 | A_96_P202044 | 4,243 | up | spermidine synthase | C:cytoplasm; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:protein binding; F:transferase activity; P:cellular process; P:secondary metabolic process |
| DB687234 | A_96_P229104 | 4,176 | up | ethphon-induced protein | C:membrane |
| AK324101 | A_96_P002246 | 4,166 | up | lipid-associated family protein | C:vacuole; C:membrane; C:plastid; C:thylakoid; C:plasma membrane |
| AK324059 | A_96_P130627 | 4,150 | up | spermidine synthase | C:cytoplasm; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:protein binding; F:transferase activity; P:cellular process; P:secondary metabolic process |
| AI486264 | A_96_P034146 | 4,116 | up | anthocyanin acyltransferase | F:transferase activity |
| TA53422_4081 | A_96_P086159 | 4,081 | up | ---NA--- | |
| U20592 | A_96_P012456 | 4,018 | up | kunitz-type protease inhibitor kpi- | F:enzyme regulator activity |
| BF051475 | A_96_P180414 | 4,009 | up | ---NA--- | |
| DB695744 | A_96_P230984 | 4,001 | up | ---NA--- | |
| AK325249 | A_96_P126582 | 3,981 | up | amino acid transporter | C:membrane; P:transport; F:transporter activity |
| AK323178 | A_96_P073874 | 3,962 | up | protein | C:vacuole; C:cell wall; F:hydrolase activity; P:catabolic process; P:lipid metabolic process |
| AK324059 | A_96_P161166 | 3,944 | up | spermidine synthase | C:cytoplasm; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:protein binding; F:transferase activity; P:cellular process; P:secondary metabolic process |
| AK321920 | A_96_P225949 | 3,860 | up | protein | F:receptor activity; C:membrane; F:catalytic activity; P:signal transduction |
| AI777019 | A_96_P136267 | 3,839 | up | ---NA--- | |
| AK319887 | A_96_P193799 | 3,825 | up | mta sah | F:binding; C:cytoplasm; P:protein modification process; F:catalytic activity; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; C:intracellular |
| AK327683 | A_96_P131862 | 3,808 | up | jasmonate zim-domain protein 1 | P:response to endogenous stimulus; P:multicellular organismal development; P:response to stress |
| AK323178 | A_96_P256527 | 3,767 | up | protein | C:vacuole; C:cell wall; F:hydrolase activity; P:catabolic process; P:lipid metabolic process |
| U37840 | A_96_P151446 | 3,727 | up | lipoxygenase | P:response to stress; P:response to endogenous stimulus; P:biological_process; P:response to biotic stimulus; P:response to external stimulus; P:metabolic process; P:growth; P:biosynthetic process; P:cellular process; P:lipid metabolic process; F:catalytic activity; C:plastid; P:response to abiotic stimulus; F:binding |
| AK324775 | A_96_P121307 | 3,699 | up | serine carboxypeptidase 1 precursor-like protein | C:extracellular region; C:peroxisome; F:hydrolase activity; P:protein metabolic process; P:catabolic process |
| AK324775 | A_96_P077159 | 3,690 | up | serine carboxypeptidase 1 precursor-like protein | C:extracellular region; C:peroxisome; F:hydrolase activity; P:protein metabolic process; P:catabolic process |

| | | | | | |
|---|---|---|---|---|---|
| BI933291 | A_96_P038801 | 3,680 | up | phenylalanine ammonia-lyase | C:cytoplasm; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process; P:biosynthetic process; F:catalytic activity; P:catabolic process |
| AK322548 | A_96_P163226 | 3,671 | up | triacylglycerol lipase | F:hydrolase activity; P:catabolic process; P:lipid metabolic process; P:cellular process |
| TA42576_4081 | A_96_P012086 | 3,671 | up | serine carboxypeptidase i precursor-like protein | C:peroxisome; F:hydrolase activity; P:protein metabolic process; P:catabolic process |
| AJ831864 | A_96_P004791 | 3,659 | up | non-specific lipid transfer protein | P:response to stress; F:lipid binding; P:transport |
| TA50399_4081 | A_96_P108637 | 3,614 | up | mate efflux family protein | P:response to biotic stimulus; C:vacuole; F:transporter activity; C:plasma membrane; P:transport; P:cellular process |
| AK321920 | A_96_P251947 | 3,597 | up | protein | F:receptor activity; C:membrane; F:catalytic activity; P:signal transduction |
| BG631358 | A_96_P194144 | 3,514 | up | PAL | |
| BF098196 | A_96_P182539 | 3,485 | up | mikc mads-box transcription factor | F:DNA binding; F:transcription factor activity; P:transcription; C:nucleoplasm |
| AW929506 | A_96_P027301 | 3,459 | up | phenylalanine ammonia-lyase | C:cytoplasm; F:catalytic activity; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process; P:catabolic process; P:biosynthetic process; P:cellular process |
| TA36040_4081 | A_96_P013091 | 3,411 | up | ---NA--- | |
| BI930270 | A_96_P204924 | 3,382 | up | ---NA--- | |
| BG626974 | A_96_P191884 | 3,378 | up | ---NA--- | |
| BG627110 | A_96_P191989 | 3,362 | up | kunitz-type protease inhibitor precursor | F:hydrolase activity; F:enzyme regulator activity |
| AK322548 | A_96_P163261 | 3,335 | up | triacylglycerol lipase | F:hydrolase activity; P:catabolic process; P:lipid metabolic process; P:cellular process |
| BI929069 | A_96_P204479 | 3,307 | up | white-brown-complex abc transporter family | P:biosynthetic process; P:cellular process; F:nucleotide binding; F:hydrolase activity; F:transporter activity; C:membrane; P:transport |
| AJ271093 | A_96_P248247 | 3,287 | up | allene oxide synthase | F:catalytic activity; F:binding; F:molecular_function |
| TA36168_4081 | A_96_P088096 | 3,274 | up | 24-sterol c-methyltransferase | P:biosynthetic process; P:DNA metabolic process; P:cell cycle; P:multicellular organismal development; P:cell growth; C:endoplasmic reticulum; F:transferase activity; P:cellular process; P:lipid metabolic process |
| U37840 | A_96_P012511 | 3,262 | up | lipoxygenase | P:response to stress; P:response to endogenous stimulus; P:biological_process; P:response to biotic stimulus; P:response to external stimulus; P:metabolic process; P:growth; P:biosynthetic process; P:cellular process; P:lipid metabolic process; F:catalytic activity; C:plastid; P:response to abiotic stimulus; F:binding |
| TA39333_4081 | A_96_P183564 | 3,249 | up | hydroxycinnamoyl- shikimate quinate hydroxycinnamoyltransferase | F:transferase activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process; P:biological_process |
| DQ399832 | A_96_P068711 | 3,249 | up | gras family transcription factor | P:transcription; P:biological_process; F:transcription factor activity; C:nucleoplasm |
| X04792 | A_96_P074929 | 3,229 | up | 1-aminocyclopropane-1-carboxylate oxidase | F:binding; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:response to stress; P:ripening; F:catalytic activity; P:metabolic process |

| | | | | | |
|---|---|---|---|---|---|
| AK324373 | A_96_P232679 | 3,228 | up | calmodulin | P:response to external stimulus; P:response to abiotic stimulus; F:signal transducer activity; P:carbohydrate metabolic process; P:cellular process; F:protein binding; P:metabolic process; F:binding; P:flower development; C:nucleus; P:biological_process; F:catalytic activity; P:signal transduction |
| AK326213 | A_96_P036741 | 3,201 | up | wax synthase | P:biosynthetic process; P:cellular process; P:lipid metabolic process |
| AK322239 | A_96_P072419 | 3,182 | up | caffeoyl- o-methyltransferase | F:transferase activity; F:nucleic acid binding; C:cytosol; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| BI209569 | A_96_P198474 | 3,155 | up | ---NA--- | |
| AK326904 | A_96_P048996 | 3,144 | up | fumarylacetoacetate hydrolase | F:hydrolase activity; P:cellular amino acid and derivative metabolic process; P:catabolic process; P:cellular process |
| AK328628 | A_96_P223994 | 3,143 | up | ---NA--- | |
| BP895928 | A_96_P106199 | 3,109 | up | nucleotidyltransferase domain containing expressed | P:transcription; F:transferase activity |
| TA38323_4081 | A_96_P131942 | 3,078 | up | protein | C:cytoplasm |
| BI209569 | A_96_P092674 | 3,073 | up | ---NA--- | |
| AW624407 | A_96_P159811 | 3,063 | up | ---NA--- | |
| AK328628 | A_96_P245675 | 3,045 | up | ---NA--- | |
| U37840 | A_96_P077809 | 3,026 | up | lipoxygenase | P:response to stress; P:response to endogenous stimulus; P:biological_process; P:response to biotic stimulus; P:response to external stimulus; P:metabolic process; P:growth; P:biosynthetic process; P:cellular process; P:lipid metabolic process; F:catalytic activity; C:plastid; P:response to abiotic stimulus; F:binding |
| AK323973 | A_96_P232094 | 3,010 | up | antocyanin acyltranspherase | F:transferase activity; P:biological_process |
| BI924266 | A_96_P189504 | 2,994 | up | ---NA--- | |
| U20592 | A_96_P258022 | 2,993 | up | kunitz-type protease inhibitor kpi- | F:enzyme regulator activity |
| AK322239 | A_96_P169739 | 2,987 | up | caffeoyl- o-methyltransferase | F:transferase activity; F:nucleic acid binding; C:cytosol; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| AI484711 | A_96_P130292 | 2,979 | up | ---NA--- | |
| TA38005_4081 | A_96_P008046 | 2,972 | up | 3-beta hydroxysteroid dehydrogenase | F:binding; P:metabolic process; F:catalytic activity |
| U37840 | A_96_P200284 | 2,953 | up | lipoxygenase | P:response to stress; P:response to endogenous stimulus; P:biological_process; P:response to biotic stimulus; P:response to external stimulus; P:metabolic process; P:growth; P:biosynthetic process; P:cellular process; P:lipid metabolic process; F:catalytic activity; C:plastid; P:response to abiotic stimulus; F:binding |
| TA55162_4081 | A_96_P120417 | 2,939 | up | protein | F:binding; F:transferase activity; P:catabolic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity; C:cytosol |
| AK328455 | A_96_P047426 | 2,932 | up | protein | F:binding; F:transferase activity; P:catabolic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity; C:cytosol |

| | | | | | |
|---|---|---|---|---|---|
| GO376336 | A_96_P186949 | 2,929 | up | jasmonate zim-domain protein 1 | P:response to endogenous stimulus; P:multicellular organismal development |
| AK328900 | A_96_P152676 | 2,927 | up | tropinone reductase | P:metabolic process; P:biological_process; F:binding; F:catalytic activity |
| BG126724 | A_96_P185909 | 2,922 | up | MYB transcription factor | F:DNA binding; P:transcription; C:nucleus |
| TA56492_4081 | A_96_P125912 | 2,907 | up | heat shock factor | F:DNA binding; P:response to stress; F:transcription factor activity; P:transcription; C:nucleoplasm |
| AK322239 | A_96_P095649 | 2,901 | up | caffeoyl- o-methyltransferase | F:transferase activity; F:nucleic acid binding; C:cytosol; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| AK322540 | A_96_P092624 | 2,877 | up | 3-beta hydroxysteroid dehydrogenase | F:binding; P:metabolic process; F:catalytic activity |
| AK322540 | A_96_P092624 | 2,877 | up | 3-beta hydroxysteroid dehydrogenase | F:binding; P:metabolic process; F:catalytic activity |
| DV935798 | A_96_P247692 | 2,874 | up | ---NA--- | ---NA--- |
| AK324283 | A_96_P217814 | 2,870 | up | chlorophyllase 2 | P:response to stress; C:vacuole; F:hydrolase activity; P:cellular process; P:catabolic process |
| BI924266 | A_96_P080319 | 2,857 | up | ---NA--- | ---NA--- |
| AK325661 | A_96_P063166 | 2,855 | up | retroelement pol polyprotein | F:nuclease activity; F:DNA binding; P:biosynthetic process; P:DNA metabolic process; F:RNA binding; P:transport; P:signal transduction; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process |
| AK321203 | A_96_P073169 | 2,854 | up | formate dehydrogenase | P:cellular process; P:lipid metabolic process; P:multicellular organismal development; F:transferase activity; F:catalytic activity; P:biosynthetic process; C:mitochondrion; P:response to abiotic stimulus; P:response to stress; P:response to external stimulus; P:metabolic process; P:cell differentiation; F:nucleotide binding; C:plastid; P:biological_process; C:membrane; C:endoplasmic reticulum; C:cell |
| AK246988 | A_96_P080209 | 2,842 | up | orn lys arg decarboxylase major region | C:plastid; F:catalytic activity |
| BF050883 | A_96_P180089 | 2,838 | up | ---NA--- | ---NA--- |
| AW092686 | A_96_P150221 | 2,825 | up | ap2 erf domain-containing transcription factor | P:response to endogenous stimulus; P:signal transduction; F:transcription factor activity; P:response to external stimulus; P:response to stress; P:response to biotic stimulus; C:nucleoplasm; P:transcription |
| AK327616 | A_96_P091739 | 2,825 | up | protein | F:binding; F:hydrolase activity; P:metabolic process; P:cellular process; P:protein modification process; C:cell |
| BG627096 | A_96_P005471 | 2,816 | up | ---NA--- | ---NA--- |
| GO376336 | A_96_P008871 | 2,801 | up | jasmonate zim-domain protein 1 | P:response to endogenous stimulus; P:multicellular organismal development |
| BG629718 | A_96_P002976 | 2,793 | up | proteinase inhibitor i | F:enzyme regulator activity; P:response to external stimulus; P:response to stress |
| AK320183 | A_96_P232574 | 2,787 | up | homogentisate -dioxygenase | P:catabolic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity; P:metabolic process; P:cellular process |

| | | | | | |
|---|---|---|---|---|---|
| AK322239 | A_96_P177399 | 2,779 | up | caffeoyl- o-methyltransferase | F:transferase activity; F:nucleic acid binding; C:cytosol; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| AK328438 | A_96_P189614 | 2,769 | up | 4-coumarate: ligase | F:nucleotide binding; F:catalytic activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| AK321203 | A_96_P166864 | 2,764 | up | formate dehydrogenase | P:cellular process; P:lipid metabolic process; P:multicellular organismal development; F:transferase activity; F:catalytic activity; P:biosynthetic process; C:mitochondrion; P:response to abiotic stimulus; P:response to stress; P:response to external stimulus; P:metabolic process; P:cell differentiation; F:nucleotide binding; C:plastid; P:biological_process; C:membrane; C:endoplasmic reticulum; C:cell |
| EG553122 | A_96_P031791 | 2,758 | up | ap2 erf domain-containing transcription factor | P:biological_process; F:DNA binding; P:transcription |
| BG126168 | A_96_P185699 | 2,757 | up | ap2 domain-containing transcription factor | P:transcription; P:anatomical structure morphogenesis; P:multicellular organismal development; P:reproduction; P:post-embryonic development; F:transcription factor activity; C:nucleoplasm |
| AK321203 | A_96_P174769 | 2,748 | up | formate dehydrogenase | P:cellular process; P:lipid metabolic process; P:multicellular organismal development; F:transferase activity; F:catalytic activity; P:biosynthetic process; C:mitochondrion; P:response to abiotic stimulus; P:response to stress; P:response to external stimulus; P:metabolic process; P:cell differentiation; F:nucleotide binding; C:plastid; P:biological_process; C:membrane; C:endoplasmic reticulum; C:cell |
| AW738087 | A_96_P164361 | 2,748 | up | hydroxycinnamoyl- shikimate quinate hydroxycinnamoyltransferase | F:transferase activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process; P:biological_process |
| AK324054 | A_96_P198409 | 2,736 | up | glucan endo- -beta-glucosidase | C:cell wall; F:binding; C:plasma membrane; C:cytoplasm; F:hydrolase activity; P:carbohydrate metabolic process; P:cellular process |
| AK321203 | A_96_P090064 | 2,734 | up | formate dehydrogenase | P:cellular process; P:lipid metabolic process; P:multicellular organismal development; F:transferase activity; F:catalytic activity; P:biosynthetic process; C:mitochondrion; P:response to abiotic stimulus; P:response to stress; P:response to external stimulus; P:metabolic process; P:cell differentiation; F:nucleotide binding; C:plastid; P:biological_process; C:membrane; C:endoplasmic reticulum; C:cell |
| AK328657 | A_96_P248802 | 2,729 | up | ---NA--- | ---NA--- |
| AK322920 | A_96_P095579 | 2,727 | up | protein | C:membrane |
| AK323867 | A_96_P081974 | 2,724 | up | annexin 11 | P:response to endogenous stimulus; P:response to stress; P:response to abiotic stimulus; C:cell; F:lipid binding; F:binding |
| BW689995 | A_96_P052621 | 2,713 | up | translocon-associated protein beta family protein | C:cytoplasm; C:plasma membrane |
| AK324283 | A_96_P036436 | 2,689 | up | chlorophyllase 2 | P:response to stress; C:vacuole; F:hydrolase activity; P:cellular process; P:catabolic process |
| DB711714 | A_96_P068811 | 2,685 | up | myosin | P:transport; P:cellular process; F:motor activity; C:cytoskeleton |
| AW625803 | A_96_P030801 | 2,680 | up | hydroxycinnamoyl- shikimate quinate hydroxycinnamoyltransferase | F:transferase activity; P:biosynthetic process |
| AK320848 | A_96_P004291 | 2,680 | up | Err:509 | P:metabolic process; F:binding; P:cellular process; F:catalytic activity |

| | | | | | |
|---|---|---|---|---|---|
| BW692061 | A_96_P035401 | 2,670 | up | ---NA--- | ---NA--- |
| AK321533 | A_96_P048951 | 2,664 | up | protein | C:membrane |
| BI922494 | A_96_P200799 | 2,661 | up | nucleic acid binding nucleotide binding | F:binding |
| AW036085 | A_96_P147161 | 2,652 | up | ---NA--- | ---NA--- |
| DB704225 | A_96_P034902 | 2,647 | up | glutathione s-transferase | F:transferase activity |
| DQ020644 | A_96_P054446 | 2,644 | up | rna polymerase ii largest subunit | F:transferase activity; C:nucleoplasm; F:DNA binding; P:transcription; C:nucleolus; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process |
| AK328438 | A_96_P219414 | 2,623 | up | 4-coumarate: ligase | F:nucleotide binding; F:catalytic activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| EF550528 | A_96_P013336 | 2,620 | up | s-adenosylmethionine decarboxylase proenzyme | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity |
| EF550528 | A_96_P013336 | 2,620 | up | s-adenosylmethionine decarboxylase proenzyme | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity |
| BT013666 | A_96_P018211 | 2,618 | up | UVH3 (ULTRAVIOLET HYPERSENSITIVE 3 | F:nuclease activity; P:response to abiotic stimulus; F:protein binding; F:DNA binding; P:response to stress; P:DNA metabolic process; C:nucleus |
| AK325264 | A_96_P077369 | 2,618 | up | cysteine protease | C:vacuole; P:transcription; F:protein binding; P:biological_process; F:transcription factor activity; F:hydrolase activity; P:protein metabolic process; P:catabolic process; P:response to endogenous stimulus; C:nucleoplasm |
| TA37090_4081 | A_96_P226024 | 2,607 | up | protein | F:transferase activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| AK328438 | A_96_P073909 | 2,607 | up | 4-coumarate: ligase | F:nucleotide binding; F:catalytic activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| BI422637 | A_96_P199644 | 2,604 | up | formate dehydrogenase | P:cellular process; P:lipid metabolic process; P:multicellular organismal development; F:transferase activity; F:catalytic activity; P:biosynthetic process; C:mitochondrion; P:response to abiotic stimulus; P:response to stress; P:response to external stimulus; P:metabolic process; P:cell differentiation; F:nucleotide binding; C:plastid; P:biological_process; C:membrane; C:endoplasmic reticulum; C:cell |
| AK327264 | A_96_P071889 | 2,601 | up | pathogen induced protein 2-4 | F:protein binding; P:response to stress; P:response to abiotic stimulus |
| AK327264 | A_96_P071889 | 2,601 | up | pathogen induced protein 2-4 | F:protein binding; P:response to stress; P:response to abiotic stimulus |
| AK319264 | A_96_P084784 | 2,597 | up | transcription factor lhy | P:response to endogenous stimulus; P:response to abiotic stimulus; P:transcription; F:DNA binding |
| DQ100158 | A_96_P044446 | 2,585 | up | senescence-inducible chloroplast stay-green protein | P:cellular process; P:catabolic process |

| | | | | | |
|---|---|---|---|---|---|
| AK328438 | A_96_P087114 | 2,578 | up | 4-coumarate: ligase | F:nucleotide binding; F:catalytic activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| AK326455 | A_96_P240072 | 2,572 | up | wrky transcription factor 1 | F:DNA binding; P:transcription; F:transcription factor activity; C:nucleoplasm |
| BI930624 | A_96_P086339 | 2,572 | up | iaa-amino acid hydrolase 3 | F:hydrolase activity; P:response to external stimulus; P:response to stress; C:cytoplasm; F:protein binding; P:protein metabolic process; P:catabolic process; C:endoplasmic reticulum; P:cellular amino acid and derivative metabolic process |
| AK321203 | A_96_P223729 | 2,568 | up | formate dehydrogenase | P:cellular process; P:lipid metabolic process; P:multicellular organismal development; F:transferase activity; F:catalytic activity; P:biosynthetic process; C:mitochondrion; P:response to abiotic stimulus; P:response to stress; P:response to external stimulus; P:metabolic process; P:cell differentiation; F:nucleotide binding; C:plastid; P:biological_process; C:membrane; C:endoplasmic reticulum; C:cell |
| EF550528 | A_96_P246422 | 2,567 | up | s-adenosylmethionine decarboxylase proenzyme | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity |
| EF550528 | A_96_P246422 | 2,567 | up | s-adenosylmethionine decarboxylase proenzyme | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity |
| BI203929 | A_96_P009751 | 2,567 | up | merozoite surface antigen | F:chitin binding; F:binding; P:chitin metabolic process; F:oxidoreductase activity; F:catalytic activity; C:extracellular region; F:FAD binding |
| AK322827 | A_96_P072959 | 2,565 | up | myo-inositol oxygenase | C:cytoplasm; F:binding; P:metabolic process; P:biosynthetic process; P:cellular process; P:carbohydrate metabolic process; P:catabolic process; P:anatomical structure morphogenesis; F:catalytic activity |
| EG553275 | A_96_P067181 | 2,563 | up | protein | P:metabolic process; F:binding; F:catalytic activity |
| TA44546_4081 | A_96_P024246 | 2,555 | up | PREDICTED: hypothetical protein [Vitis vinifera] | C:plastid |
| DB684196 | A_96_P050686 | 2,524 | up | alpha-l-fucosidase 2 | C:cytoplasm |
| TA41641_4081 | A_96_P098909 | 2,521 | up | metallocarboxypeptidase inhibitor precursor | F:enzyme regulator activity |
| AK246404 | A_96_P020991 | 2,519 | up | polcalcin jun o 2 | F:catalytic activity; C:mitochondrion; C:nucleus; F:binding; P:response to stress; P:response to abiotic stimulus |
| BF052164 | A_96_P180739 | 2,519 | up | ---NA--- | |
| AK323867 | A_96_P074459 | 2,517 | up | annexin 11 | P:response to endogenous stimulus; P:response to stress; P:response to abiotic stimulus; C:cell; F:lipid binding; F:binding |
| BE433649 | A_96_P173309 | 2,512 | up | formate dehydrogenase | P:cellular process; P:lipid metabolic process; P:multicellular organismal development; F:transferase activity; F:catalytic activity; P:biosynthetic process; C:mitochondrion; P:response to abiotic stimulus; P:response to stress; P:response to external stimulus; P:metabolic process; P:cell differentiation; F:nucleotide binding; C:plastid; P:biological_process; C:membrane; C:endoplasmic reticulum; C:cell |
| TA40663_4081 | A_96_P175004 | 2,512 | up | nad h isoflavone reductase | P:biological_process; P:metabolic process; F:catalytic activity; C:mitochondrion |
| GO372514 | A_96_P074544 | 2,505 | up | fer_solly ame: full=ferredoxin | P:protein metabolic process; P:cellular process; F:binding; F:molecular_function; P:transport; C:plastid; P:generation of precursor metabolites and energy |

| | | | | | |
|---|---|---|---|---|---|
| AK247122 | A_96_P078114 | 2,504 | up | ethylene-regulated transcript 2 | C:nucleus |
| DB698684 | A_96_P070694 | 2,503 | up | protein | F:transferase activity; P:cellular process; C:membrane |
| BM410619 | A_96_P073764 | 2,490 | up | flavonol synthase flavanone 3- | P:metabolic process; F:catalytic activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| DB686099 | A_96_P044581 | 2,480 | up | soul heme-binding family protein | C:cytoplasm; C:vacuole; F:binding; C:plasma membrane |
| AW625830 | A_96_P160556 | 2,479 | up | saur33 - auxin-responsive saur family member | P:response to endogenous stimulus; P:signal transduction; C:plastid; C:mitochondrion; F:protein binding |
| TA51698_4081 | A_96_P110042 | 2,478 | up | ---NA--- | ---NA--- |
| DB725057 | A_96_P244450 | 2,477 | up | ---NA--- | ---NA--- |
| AK328540 | A_96_P090769 | 2,471 | up | flc-like 1 splice variant 4 | F:DNA binding; P:transcription |
| AK328540 | A_96_P090769 | 2,471 | up | flc-like 1 splice variant 4 | F:DNA binding; P:transcription |
| AK322251 | A_96_P229534 | 2,468 | up | transcription initiation factor iib | F:translation factor activity, nucleic acid binding; F:binding; P:cellular component organization; P:transcription; F:protein binding; F:transcription regulator activity; C:ribosome; P:translation; C:nucleoplasm |
| DY523456 | A_96_P086424 | 2,464 | up | cytochrome p450 | C:cytoplasm; F:molecular_function; F:binding; P:metabolic process; F:oxygen binding; F:catalytic activity |
| AK327264 | A_96_P093959 | 2,452 | up | pathogen induced protein 2-4 | F:protein binding; P:response to stress; P:response to abiotic stimulus |
| AK327264 | A_96_P093959 | 2,452 | up | pathogen induced protein 2-4 | F:protein binding; P:response to stress; P:response to abiotic stimulus |
| BI206372 | A_96_P022421 | 2,443 | up | ---NA--- | ---NA--- |
| BI924417 | A_96_P201779 | 2,440 | up | MAP3K delta-1 protein kinase | P:signal transduction; C: membrane; |
| AK324075 | A_96_P011461 | 2,438 | up | ac007591_30 ests gb | C:plastid; P:transport |
| GO374313 | A_96_P036721 | 2,438 | up | cytochrome p450 | F:binding; P:metabolic process; F:catalytic activity; F:molecular_function |
| AK322540 | A_96_P141617 | 2,437 | up | 3-beta hydroxysteroid dehydrogenase | F:binding; P:metabolic process; F:catalytic activity |
| AK322540 | A_96_P141617 | 2,437 | up | 3-beta hydroxysteroid dehydrogenase | F:binding; P:metabolic process; F:catalytic activity |
| X98308 | A_96_P012666 | 2,437 | up | myb-related transcription factor | P:response to endogenous stimulus; F:DNA binding; P:biological_process; P:transcription; C:nucleus; P:response to stress; P:response to abiotic stimulus |
| BG132464 | A_96_P189084 | 2,433 | up | atp synthase beta subunit | C:membrane; C:intracellular; P:transport; P:biosynthetic process; P:generation of precursor metabolites and energy; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; P:cellular process; F:hydrolase activity; F:transporter activity; F:nucleotide binding; C:plastid; C:thylakoid |

| | | | | | |
|---|---|---|---|---|---|
| BI207077 | A_96_P078309 | 2,432 | up | isoform a | F:chitin binding; P:chitin metabolic process; F:oxidoreductase activity; F:catalytic activity; C:extracellular region; F:FAD binding |
| AK321203 | A_96_P144796 | 2,431 | up | formate dehydrogenase | P:cellular process; P:lipid metabolic process; P:multicellular organismal development; F:transferase activity; F:catalytic activity; P:biosynthetic process; C:mitochondrion; P:response to abiotic stimulus; P:response to stress; P:response to external stimulus; P:metabolic process; P:cell differentiation; F:nucleotide binding; C:plastid; P:biological_process; C:membrane; C:endoplasmic reticulum; C:cell |
| BI209348 | A_96_P198349 | 2,429 | up | jasmonate ZIM-domain protein 3 | P:response to endogenous stimulus; P:signal transduction; P:response to stress; P:response to biotic stimulus |
| X55688 | A_96_P260327 | 2,426 | up | ---NA--- | ---NA--- |
| X94946 | A_96_P013446 | 2,424 | up | trypsin proteinase inhibitor precursor | F:enzyme regulator activity |
| AK319494 | A_96_P194224 | 2,420 | up | thioredoxin m | P:multicellular organismal development; F:enzyme regulator activity; P:cellular homeostasis; C:plastid; P:biological_process; P:cell communication |
| AK322548 | A_96_P048842 | 2,417 | up | triacylglycerol lipase | F:hydrolase activity; P:catabolic process; P:lipid metabolic process; P:cellular process |
| BI931500 | A_96_P205414 | 2,416 | up | ---NA--- | ---NA--- |
| AW928869 | A_96_P165351 | 2,413 | up | ---NA--- | ---NA--- |
| AK324503 | A_96_P005516 | 2,411 | up | esterase lipase thioesterase family protein | F:hydrolase activity |
| BG124969 | A_96_P185104 | 2,410 | up | ac transposase | F:binding |
| AK324503 | A_96_P168554 | 2,405 | up | esterase lipase thioesterase family protein | F:hydrolase activity |
| TA37423_4081 | A_96_P135647 | 2,401 | up | glutathione s-transferase | P:response to endogenous stimulus; P:signal transduction; F:catalytic activity; F:transferase activity; P:response to stress; P:cellular amino acid and derivative metabolic process; P:carbohydrate metabolic process; P:metabolic process; P:cellular process |
| AK321203 | A_96_P001021 | 2,400 | up | formate dehydrogenase | P:cellular process; P:lipid metabolic process; P:multicellular organismal development; F:transferase activity; F:catalytic activity; P:biosynthetic process; C:mitochondrion; P:response to abiotic stimulus; P:response to stress; P:response to external stimulus; P:metabolic process; P:cell differentiation; F:nucleotide binding; C:plastid; P:biological_process; C:membrane; C:endoplasmic reticulum; C:cell |
| BE463214 | A_96_P225704 | 2,400 | up | non-specific lipid transfer protein | P:response to stress; F:lipid binding; P:transport |
| AW623365 | A_96_P159011 | 2,398 | up | formate dehydrogenase | P:cellular process; P:lipid metabolic process; P:multicellular organismal development; F:transferase activity; F:catalytic activity; P:biosynthetic process; C:mitochondrion; P:response to abiotic stimulus; P:response to stress; P:response to external stimulus; P:metabolic process; P:cell differentiation; F:nucleotide binding; C:plastid; P:biological_process; C:membrane; C:endoplasmic reticulum; C:cell |
| AW222833 | A_96_P154656 | 2,396 | up | Polyprotein, putative [Solanum demissum] | F:binding |
| BE463214 | A_96_P014526 | 2,392 | up | non-specific lipid transfer protein | P:response to stress; F:lipid binding; P:transport |

| | | | | | |
|---|---|---|---|---|---|
| DB696712 | A_96_P011036 | 2,390 | up | iaa-amino acid hydrolase ilr1 | P:response to external stimulus; P:response to stress; C:endoplasmic reticulum; F:hydrolase activity; P:protein metabolic process; P:catabolic process; F:protein binding; P:cellular amino acid and derivative metabolic process |
| L21194 | A_96_P013031 | 2,385 | up | trypsin proteinase inhibitor precursor | F:enzyme regulator activity |
| TA51528_4081 | A_96_P109807 | 2,381 | up | ---NA--- | ---NA--- |
| BP884283 | A_96_P212334 | 2,380 | up | ---NA--- | ---NA--- |
| BI924398 | A_96_P201764 | 2,373 | up | zinc ion binding | C:intracellular; F:binding |
| Z46674 | A_96_P077614 | 2,370 | up | hypothetical protein Pmar_PMAR016250 | F:oxidoreductase activity; F:catalytic activity; F:FAD binding |
| TA37090_4081 | A_96_P081119 | 2,365 | up | protein | F:transferase activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| EF550528 | A_96_P135597 | 2,360 | up | s-adenosylmethionine decarboxylase proenzyme | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity |
| EF550528 | A_96_P135597 | 2,360 | up | s-adenosylmethionine decarboxylase proenzyme | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity |
| AK327264 | A_96_P249857 | 2,349 | up | pathogen induced protein 2-4 | F:protein binding; P:response to stress; P:response to abiotic stimulus |
| AK327264 | A_96_P249857 | 2,349 | up | pathogen induced protein 2-4 | F:protein binding; P:response to stress; P:response to abiotic stimulus |
| AY157061 | A_96_P013301 | 2,348 | up | protein | F:DNA binding; P:transcription; F:transcription factor activity; C:nucleoplasm |
| AY157061 | A_96_P013301 | 2,348 | up | protein | F:DNA binding; P:transcription; F:transcription factor activity; C:nucleoplasm |
| AI489536 | A_96_P132342 | 2,348 | up | pectin methylesterase-like protein | F:hydrolase activity; P:cellular component organization; P:cellular process; C:cell wall; F:enzyme regulator activity; C:plastid; C:plasma membrane; P:carbohydrate metabolic process |
| BI924270 | A_96_P201664 | 2,347 | up | ---NA--- | |
| BF052063 | A_96_P180679 | 2,345 | up | ---NA--- | |
| EG553672 | A_96_P081084 | 2,342 | up | protease inhibitor seed storage lipid transfer protein family protein | C:cell; P:transport; F:lipid binding; F:hydrolase activity |
| AK321000 | A_96_P110407 | 2,342 | up | ap2 erf domain-containing transcription factor | F:binding; P:transport; P:cellular process; P:multicellular organismal development; P:reproduction; P:post-embryonic development; P:embryonic development; P:transcription |
| AK323867 | A_96_P140702 | 2,334 | up | annexin 11 | P:response to endogenous stimulus; P:response to stress; P:response to abiotic stimulus; C:cell; F:lipid binding; F:binding |
| AK323773 | A_96_P231649 | 2,331 | up | ---NA--- | |
| AK327264 | A_96_P254337 | 2,327 | up | pathogen induced protein 2-4 | F:protein binding; P:response to stress; P:response to abiotic stimulus |

| | | | | | |
|---|---|---|---|---|---|
| AK320730 | A_96_P079544 | 2,326 | up | protein | F:hydrolase activity; P:carbohydrate metabolic process; C:cytoplasm; F:binding |
| DB713387 | A_96_P068101 | 2,318 | up | transcription initiation | C:nucleoplasm; F:translation factor activity, nucleic acid binding; F:transcription regulator activity; P:cellular component organization; P:transcription; C:ribosome; P:translation |
| AK319191 | A_96_P249807 | 2,317 | up | Calmodulin binding | P:response to stress |
| AK326809 | A_96_P236723 | 2,315 | up | protein | F:molecular_function; P:biological_process; C:cellular_component |
| GO372355 | A_96_P012236 | 2,314 | up | ---NA--- | ---NA--- |
| AK326022 | A_96_P251942 | 2,311 | up | embryo-abundant protein | P:biological_process; P:metabolic process; C:mitochondrion; F:transferase activity |
| BE354492 | A_96_P171554 | 2,301 | up | small basic intrinsic protein 1 | C:membrane |
| AJ786362 | A_96_P021271 | 2,298 | up | protein | C:cytoplasm; P:biosynthetic process; P:cellular process; P:lipid metabolic process; F:catalytic activity; P:biological_process; P:metabolic process; C:membrane; P:response to abiotic stimulus |
| BT014086 | A_96_P143966 | 2,296 | up | protein | F:binding |
| BI203201 | A_96_P062701 | 2,291 | up | branched-chain-amino-acid aminotransferase chloroplast expressed | F:transferase activity; C:plastid; P:catabolic process; P:cellular amino acid and derivative metabolic process; P:biosynthetic process |
| AK319296 | A_96_P060166 | 2,290 | up | ---NA--- | |
| GO374941 | A_96_P167039 | 2,287 | up | spermidine synthase | F:transferase activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:cellular process; P:secondary metabolic process |
| BG123989 | A_96_P106709 | 2,282 | up | abhydrolase domain | F:hydrolase activity |
| TA53412_4081 | A_96_P113112 | 2,282 | up | phytosulfokine peptide precursor | P:cell growth; F:receptor binding; C:extracellular region; P:signal transduction; P:cellular process |
| AI488257 | A_96_P005126 | 2,278 | up | non-specific lipid transfer protein | P:response to stress; F:lipid binding; P:transport |
| TA54771_4081 | A_96_P118882 | 2,275 | up | unconventional myosin | F:signal transducer activity; F:protein binding; C:cytoskeleton; F:nucleotide binding; P:signal transduction; F:motor activity; C:membrane |
| AK319494 | A_96_P007246 | 2,269 | up | thioredoxin m | P:multicellular organismal development; F:enzyme regulator activity; P:cellular homeostasis; C:plastid; P:biological_process; P:cell communication |
| TA54720_4081 | A_96_P118667 | 2,269 | up | 60s ribosomal protein l37 | F:binding; F:structural molecule activity; P:cellular process; C:ribosome; C:cytosol; P:translation; F:RNA binding |
| BM408622 | A_96_P207524 | 2,262 | up | ---NA--- | ---NA--- |
| AK320730 | A_96_P148821 | 2,262 | up | protein | F:hydrolase activity; P:carbohydrate metabolic process; C:cytoplasm; F:binding |
| AK320730 | A_96_P148821 | 2,262 | up | protein | F:hydrolase activity; P:carbohydrate metabolic process; C:cytoplasm; F:binding |
| BI925451 | A_96_P202414 | 2,252 | up | ---NA--- | ---NA--- |
| BG127874 | A_96_P186484 | 2,252 | up | amp dependent | C:peroxisome; F:catalytic activity; F:nucleotide binding; P:biosynthetic process; P:cellular process |
| TA40663_4081 | A_96_P097479 | 2,251 | up | nad h isoflavone reductase | P:biological_process; P:metabolic process; F:catalytic activity; C:mitochondrion |

| | | | | | |
|---|---|---|---|---|---|
| BG628739 | A_96_P192939 | 2,250 | up | unnamed protein product [Vitis vinifera] | ---NA--- |
| AK323545 | A_96_P073749 | 2,247 | up | 4-coumarate: ligase | F:nucleotide binding; F:catalytic activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| DV105470 | A_96_P247362 | 2,246 | up | mip tip subfamily | P:transport; P:cellular process; C:membrane; F:transporter activity |
| AK326213 | A_96_P080914 | 2,246 | up | wax synthase | P:biosynthetic process; P:cellular process; P:lipid metabolic process |
| AK320112 | A_96_P184129 | 2,238 | up | hypothetical protein ARALYDRAFT_493280 | C:plastid |
| AW738593 | A_96_P047556 | 2,237 | up | protein | P:cellular amino acid and derivative metabolic process; P:secondary metabolic process; F:protein binding; C:cytoplasm; F:hydrolase activity; F:transcription factor activity; P:signal transduction; P:response to endogenous stimulus; P:response to abiotic stimulus; P:post-embryonic development; P:metabolic process; P:cellular process; P:protein modification process; C:cell; C:nucleoplasm; P:transcription |
| AK324790 | A_96_P118107 | 2,231 | up | f-box protein | F:protein binding |
| AW933342 | A_96_P168394 | 2,226 | up | ---NA--- | ---NA--- |
| AK224831 | A_96_P068621 | 2,225 | up | hydrogen peroxide-induced | P:biological_process; P:response to stress |
| AK224831 | A_96_P068621 | 2,225 | up | hydrogen peroxide-induced 1 | P:biological_process; P:response to stress |
| AK320768 | A_96_P005781 | 2,222 | up | jasmonate zim-domain protein 1 | P:response to endogenous stimulus; P:signal transduction; P:response to stress; P:response to biotic stimulus |
| DV105669 | A_96_P025741 | 2,221 | up | major latex-like protein | P:response to biotic stimulus; P:defense response; F:molecular_function; C:cellular_component |
| BI930624 | A_96_P167124 | 2,220 | up | iaa-amino acid hydrolase 3 | F:hydrolase activity; P:response to external stimulus; P:response to stress; C:cytoplasm; F:protein binding; P:protein metabolic process; P:catabolic process; C:endoplasmic reticulum; P:cellular amino acid and derivative metabolic process |
| BM410619 | A_96_P050431 | 2,220 | up | flavonol synthase flavanone 3- | P:metabolic process; F:catalytic activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| BP893968 | A_96_P062311 | 2,216 | up | apetala 2-like protein | F:transcription factor activity; P:transcription; C:nucleoplasm |
| AW455314 | A_96_P157461 | 2,215 | up | triacylglycerol lipase | F:hydrolase activity; C:mitochondrion; P:catabolic process; P:lipid metabolic process; P:cellular process |
| AJ785553 | A_96_P142971 | 2,214 | up | ---NA--- | ---NA--- |
| BP881758 | A_96_P211384 | 2,204 | up | cytochrome p450 | F:binding; P:metabolic process; F:molecular_function; F:catalytic activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| TA55208_4081 | A_96_P120607 | 2,202 | up | wd40-repeat protein | C:intracellular; F:nucleotide binding |
| AK320937 | A_96_P097614 | 2,192 | up | acetyl- c-acetyltransferase | C:peroxisome; F:transferase activity; P:reproduction; P:post-embryonic development; P:embryonic development; P:metabolic process; P:cellular process; P:catabolic process; P:cellular amino acid and derivative metabolic process; P:biosynthetic process; P:lipid metabolic process |
| BP893397 | A_96_P216099 | 2,190 | up | ---NA--- | ---NA--- |

| | | | | | |
|---|---|---|---|---|---|
| CV582087 | A_96_P226939 | 2,186 | up | ---NA--- | ---NA--- |
| AK323579 | A_96_P095189 | 2,185 | up | c- sterol isomerase | F:catalytic activity; C:membrane; C:endoplasmic reticulum; P:biosynthetic process; P:cellular process; P:lipid metabolic process |
| AK327264 | A_96_P093954 | 2,181 | up | pathogen induced protein 2-4 | F:protein binding; P:response to stress; P:response to abiotic stimulus |
| BP908167 | A_96_P221559 | 2,181 | up | WD-40 repeat family protein | C:intracellular; C:membrane |
| DB720921 | A_96_P241539 | 2,180 | up | ---NA--- | ---NA--- |
| AK320730 | A_96_P163111 | 2,180 | up | protein | F:hydrolase activity; P:carbohydrate metabolic process; C:cytoplasm; F:binding |
| BE462099 | A_96_P060771 | 2,176 | up | tetracycline transporter | C:cytoplasm; F:transporter activity; C:membrane; P:transport |
| CK714819 | A_96_P226034 | 2,170 | up | abhydrolase domain | F:hydrolase activity |
| AY093595 | A_96_P012701 | 2,168 | up | osmotin-like protein | P:response to stress; P:response to biotic stimulus; C:vacuole; P:biological_process |
| AJ785388 | A_96_P142786 | 2,167 | up | ---NA--- | ---NA--- |
| AK328540 | A_96_P172114 | 2,166 | up | flc-like 1 splice variant 4 | F:DNA binding; P:transcription |
| AK328540 | A_96_P172114 | 2,166 | up | flc-like 1 splice variant 4 | F:DNA binding; P:transcription |
| BG129200 | A_96_P187154 | 2,165 | up | Polyprotein, putative [Solanum demissum] | F:nucleic acid binding |
| AY157061 | A_96_P232429 | 2,164 | up | protein | F:DNA binding; P:transcription; F:transcription factor activity; C:nucleoplasm |
| AY157061 | A_96_P232429 | 2,164 | up | protein | F:DNA binding; P:transcription; F:transcription factor activity; C:nucleoplasm |
| AK323147 | A_96_P025976 | 2,163 | up | cytochrome p450 | C:cell; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process; F:catalytic activity; C:plasma membrane; F:molecular_function; F:binding; P:metabolic process; C:membrane; C:endoplasmic reticulum |
| DV104023 | A_96_P246562 | 2,160 | up | cgi-144-like protein | C:cytoplasm |
| TA43443_4081 | A_96_P180214 | 2,158 | up | pre-rrna-processing protein | C:nucleus; C:cytosol |
| AK323967 | A_96_P114742 | 2,158 | up | protein | F:enzyme regulator activity |
| AK224691 | A_96_P072804 | 2,155 | up | jasmonate ZIM-domain protein 3 | P:response to endogenous stimulus; P:signal transduction; P:response to stress; P:response to biotic stimulus |
| BE431646 | A_96_P171999 | 2,155 | up | cinnamic acid 4-hydroxylase | F:binding; F:catalytic activity; P:metabolic process; F:molecular_function; P:cellular amino acid and derivative metabolic process; P:biosynthetic process; P:secondary metabolic process |
| BI206635 | A_96_P025971 | 2,151 | up | inorganic pyrophosphatase | F:hydrolase activity; C:membrane; C:cytoplasm; C:nucleus; F:binding; P:generation of precursor metabolites and energy |
| TA53991_4081 | A_96_P115502 | 2,149 | up | jasmonate ZIM-domain protein 1 | P:response to endogenous stimulus; P:signal transduction; P:response to stress; P:response to biotic stimulus |
| AK320874 | A_96_P056516 | 2,149 | up | cytochrome p450 | P:metabolic process; F:binding; F:catalytic activity |

| | | | | | |
|---|---|---|---|---|---|
| TA37419_4081 | A_96_P076119 | 2,147 | up | spermidine synthase | F:transferase activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:cellular process; P:secondary metabolic process |
| BW688800 | A_96_P067501 | 2,144 | up | PREDICTED: hypothetical protein [Vitis vinifera] | C:mitochondrion; F:molecular_function; P:biological_process |
| BI931368 | A_96_P073684 | 2,144 | up | lactoylglutathione lyase | F:lyase activity |
| BT012906 | A_96_P015157 | 2,144 | up | annexin 3 | P:response to stress; P:response to abiotic stimulus; C:cell; F:lipid binding; F:binding |
| AW443860 | A_96_P060181 | 2,141 | up | protein binding | F:nucleic acid binding; C:cytoplasm; P:cell cycle; F:nucleotide binding; P:chromosome segregation; P:cell division; C:cellular_component |
| BI207682 | A_96_P197724 | 2,140 | up | late blight resistance identical | P:cell death; P:response to stress; F:nucleotide binding |
| EF550528 | A_96_P175496 | 2,139 | up | s-adenosylmethionine decarboxylase proenzyme | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity |
| EF550528 | A_96_P175496 | 2,139 | up | s-adenosylmethionine decarboxylase proenzyme | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity |
| AK323093 | A_96_P020541 | 2,136 | up | hydrophobic low temperature and salt responsive | P:response to stress; P:response to abiotic stimulus; C:cell; C:cytoplasm; P:response to biotic stimulus; C:membrane |
| BT014086 | A_96_P019271 | 2,134 | up | protein | F:binding |
| BT014086 | A_96_P019271 | 2,134 | up | protein | F:binding |
| AI777386 | A_96_P136542 | 2,133 | up | ---NA--- | ---NA--- |
| AK224691 | A_96_P183619 | 2,133 | up | jasmonate ZIM-domain protein 3 | P:response to endogenous stimulus; P:signal transduction; P:response to stress; P:response to biotic stimulus |
| BI925811 | A_96_P202634 | 2,128 | up | cinnamate 4-hydroxylase | F:binding; F:catalytic activity; P:metabolic process; F:molecular_function; P:cellular amino acid and derivative metabolic process; P:biosynthetic process; P:secondary metabolic process |
| AK322781 | A_96_P181229 | 2,124 | up | auxin-responsive protein | P:transcription; F:protein binding; P:response to endogenous stimulus; P:signal transduction; C:nucleus |
| AK328581 | A_96_P121587 | 2,124 | up | auxin-induced protein | P:catabolic process; P:cellular process; P:secondary metabolic process; C:cytoplasm; F:transferase activity; P:response to stress; P:response to biotic stimulus; P:response to endogenous stimulus; P:signal transduction; F:catalytic activity; P:cellular amino acid and derivative metabolic process |
| AI485590 | A_96_P130647 | 2,121 | up | cucumber peeling | C:cytoplasmic membrane-bounded vesicle; F:electron carrier activity; F:copper ion binding |
| BG135405 | A_96_P190979 | 2,120 | up | ---NA-- | ---NA--- |
| AK325600 | A_96_P060576 | 2,117 | up | cytochrome p450 | F:binding; P:metabolic process; F:molecular_function; F:catalytic activity |
| BW690986 | A_96_P035461 | 2,111 | up | ---NA--- | ---NA--- |
| BE354194 | A_96_P171304 | 2,110 | up | ---NA--- | ---NA--- |

| | | | | | |
|---|---|---|---|---|---|
| ES893872 | A_96_P074162 | 2,107 | up | glutathione s-transferase | P:response to endogenous stimulus; P:signal transduction; F:catalytic activity; F:transferase activity; P:response to stress; P:cellular amino acid and derivative metabolic process; P:carbohydrate metabolic process; P:metabolic process; P:cellular process |
| BP886864 | A_96_P213349 | 2,106 | up | atp-binding cassette superfamily | P:biosynthetic process; P:cellular process; F:hydrolase activity; F:transporter activity; C:plasma membrane; F:nucleotide binding; P:transport |
| AK320297 | A_96_P131162 | 2,105 | up | phosphatidylcholine-sterol o-acyltransferase | C:cytoplasm; F:transferase activity; P:lipid metabolic process; P:biosynthetic process |
| AW648198 | A_96_P161221 | 2,103 | up | ---NA--- | ---NA--- |
| AK319314 | A_96_P104819 | 2,101 | up | ---NA--- | ---NA--- |
| BG125803 | A_96_P185524 | 2,100 | up | ---NA--- | ---NA--- |
| AK325661 | A_96_P211769 | 2,097 | up | retroelement pol polyprotein | F:nuclease activity; F:DNA binding; P:biosynthetic process; P:DNA metabolic process; F:RNA binding; P:transport; P:signal transduction; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process |
| AK325661 | A_96_P211769 | 2,097 | up | retroelement pol polyprotein | F:nuclease activity; F:DNA binding; P:biosynthetic process; P:DNA metabolic process; F:RNA binding; P:transport; P:signal transduction; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process |
| BG628279 | A_96_P005836 | 2,096 | up | ---NA--- | ---NA--- |
| AK322846 | A_96_P230219 | 2,096 | up | ---NA--- | ---NA--- |
| AK224691 | A_96_P088314 | 2,094 | up | jasmonate ZIM-domain protein 3 | P:response to endogenous stimulus; P:signal transduction; P:response to stress; P:response to biotic stimulus |
| DB684300 | A_96_P059491 | 2,093 | up | leucine rich | C:cytoplasm; F:kinase activity |
| TA56609_4081 | A_96_P126392 | 2,091 | up | ---NA--- | ---NA--- |
| AI774144 | A_96_P134882 | 2,088 | up | calcium binding protein | F:binding |
| AK322540 | A_96_P184944 | 2,087 | up | 3-beta hydroxysteroid dehydrogenase | F:binding; P:metabolic process; F:catalytic activity |
| AK322540 | A_96_P184944 | 2,087 | up | 3-beta hydroxysteroid dehydrogenase | F:binding; P:metabolic process; F:catalytic activity |
| BI922284 | A_96_P200704 | 2,084 | up | relative to apetala2 1 | P:transcription |
| AK329228 | A_96_P116807 | 2,083 | up | zinc ion binding protein | F:zinc ion binding; P:biological_process; F:protein binding; C:cellular_component |
| AW933248 | A_96_P168299 | 2,081 | up | late embryogenesis abundant domain-containing protein | P:response to stress; P:response to biotic stimulus; P:reproduction; P:post-embryonic development; P:embryonic development; P:response to abiotic stimulus; P:response to external stimulus |
| DV104534 | A_96_P246722 | 2,080 | up | protein | C:cytoplasm; C:membrane; C:cell wall; C:Golgi apparatus; P:transport; C:plasma membrane |

| | | | | | |
|---|---|---|---|---|---|
| AK319313 | A_96_P245365 | 2,074 | up | protein | P:protein modification process; F:nucleotide binding; C:cytosol; F:kinase activity; P:cellular amino acid and derivative metabolic process |
| AK224831 | A_96_P087869 | 2,074 | up | hydrogen peroxide-induced | P:biological_process; P:response to stress |
| AK224831 | A_96_P087869 | 2,074 | up | hydrogen peroxide-induced 1 | P:biological_process; P:response to stress |
| TA53354_4081 | A_96_P112857 | 2,073 | up | eix receptor 2 | F:receptor activity; F:protein binding; P:signal transduction |
| GO374941 | A_96_P091449 | 2,073 | up | spermidine synthase | F:transferase activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:cellular process; P:secondary metabolic process |
| GO374941 | A_96_P091449 | 2,073 | up | spermidine synthase | F:transferase activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:cellular process; P:secondary metabolic process |
| CD003339 | A_96_P225579 | 2,072 | up | ---NA--- | ---NA--- |
| BI934065 | A_96_P065106 | 2,067 | up | late embryogenesis abundant domain-containing protein | P:response to stress; P:response to biotic stimulus; P:reproduction; P:post-embryonic development; P:embryonic development; P:response to abiotic stimulus; P:response to external stimulus |
| AK320730 | A_96_P025531 | 2,063 | up | protein | F:hydrolase activity; P:carbohydrate metabolic process; C:cytoplasm; F:binding |
| TA48642_4081 | A_96_P026291 | 2,061 | up | lipid binding protein | C:cytoplasm; C:mitochondrion |
| BG643246 | A_96_P005226 | 2,059 | up | vacuolar sorting receptor 1 | P:transport; P:cellular process; F:receptor activity; C:plasma membrane; C:cytoplasm; F:binding; C:Golgi apparatus; C:plastid; P:signal transduction |
| AI898304 | A_96_P140962 | 2,058 | up | nucleic acid binding | F:DNA binding; P:transcription |
| GO374941 | A_96_P000096 | 2,058 | up | spermidine synthase | F:transferase activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:cellular process; P:secondary metabolic process |
| BG631546 | A_96_P004906 | 2,057 | up | family ii extracellular lipase 1 | F:transferase activity; C:extracellular region; P:reproduction; F:hydrolase activity; P:biosynthetic process; P:catabolic process; P:lipid metabolic process |
| BP897734 | A_96_P217894 | 2,054 | up | 14-3-3 protein | F:protein binding |
| TA55705_4081 | A_96_P122577 | 2,051 | up | ---NA--- | ---NA--- |
| TA55711_4081 | A_96_P122602 | 2,051 | up | ---NA--- | ---NA--- |
| TA53364_4081 | A_96_P112897 | 2,046 | up | udp-glucose:flavonoid glucoside - glucosyltransferase | P:metabolic process; F:transferase activity |
| BP893656 | A_96_P216174 | 2,044 | up | ---NA--- | ---NA--- |
| AW625828 | A_96_P034406 | 2,043 | up | saur33 - auxin-responsive saur family member | P:response to endogenous stimulus; P:signal transduction; C:plastid; C:mitochondrion; F:protein binding |
| AK325632 | A_96_P128122 | 2,043 | up | lipase class 3 family protein | F:hydrolase activity; C:membrane; P:catabolic process; P:lipid metabolic process; P:cellular process |
| BG791295 | A_96_P196029 | 2,043 | up | protein | F:transferase activity; P:metabolic process |

| | | | | | |
|---|---|---|---|---|---|
| AK325559 | A_96_P043126 | 2,041 | up | f-box kelch repeat-containing f-box family protein | C:membrane |
| BI925709 | A_96_P202594 | 2,041 | up | ---NA--- | ---NA--- |
| DB712076 | A_96_P069879 | 2,039 | up | vacuolar sorting | P:transport; P:cellular process |
| CD003017 | A_96_P225269 | 2,037 | up | ---NA--- | ---NA--- |
| BI205334 | A_96_P086144 | 2,035 | up | gh3 family protein | F:catalytic activity; P:biological_process; P:response to endogenous stimulus |
| AK322664 | A_96_P060356 | 2,033 | up | hd domain class transcription factor | P:response to endogenous stimulus; P:signal transduction; P:response to stress; P:response to abiotic stimulus; P:transcription; F:DNA binding; F:transcription regulator activity; F:protein binding; F:transcription factor activity; C:nucleoplasm |
| BG131595 | A_96_P119432 | 2,030 | up | pentatricopeptide repeat-containing | C:plastid; C:mitochondrion |
| AW094391 | A_96_P150926 | 2,029 | up | SDM1 | P:cellular process; F:nucleotide binding; F:motor activity; C:cytoskeleton |
| BI926661 | A_96_P203209 | 2,024 | up | atp binding | C:cytoplasm |
| BP892917 | A_96_P215939 | 2,021 | up | 3-beta hydroxysteroid dehydrogenase | P:metabolic process; F:catalytic activity; F:binding |
| TA41248_4081 | A_96_P079089 | 2,020 | up | protein phosphatase 2c | P:response to endogenous stimulus; P:signal transduction; P:protein modification process; P:response to stress; P:response to abiotic stimulus; F:binding; C:cell; P:cellular process; F:hydrolase activity; F:protein binding |
| AK330010 | A_96_P046946 | 2,019 | up | esterase lipase thioesterase family protein | F:catalytic activity |
| AK319742 | A_96_P052056 | 2,014 | up | cxe carboxylesterase | P:metabolic process; F:hydrolase activity |
| FS195438 | A_96_P125062 | 2,013 | up | protein | F:RNA binding; C:mitochondrion; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; F:nucleotide binding |
| AK330010 | A_96_P198639 | 2,012 | up | esterase lipase thioesterase family protein | F:catalytic activity |
| AK321499 | A_96_P078104 | 2,012 | up | protein | P:biosynthetic process; P:cellular process; P:protein modification process; F:nucleotide binding; F:kinase activity; P:cellular amino acid and derivative metabolic process |
| AK319311 | A_96_P080504 | 2,010 | up | casein kinase | P:biosynthetic process; P:cellular process; P:protein modification process; F:nucleotide binding; F:kinase activity; C:plastid; C:mitochondrion; P:cellular amino acid and derivative metabolic process |
| BM411460 | A_96_P240283 | 2,010 | up | phenylcoumaran benzylic ether reductase 3 | P:metabolic process; F:catalytic activity; C:cytoplasm; F:binding |
| BM411358 | A_96_P208129 | 2,010 | up | ---NA--- | ---NA--- |
| BT013144 | A_96_P182679 | 2,008 | up | rubisco subunit binding-protein alpha subunit | P:protein metabolic process; P:cellular process; P:biosynthetic process; C:mitochondrion; C:plastid; P:cellular component organization; F:nucleotide binding; P:embryonic development; F:protein binding; C:ribosome; C:membrane; C:extracellular region |

| | | | | | |
|---|---|---|---|---|---|
| AK324517 | A_96_P232794 | 2,006 | up | anthocyanin 5-o-glucosyltransferase | F:transferase activity; P:metabolic process |
| DB694696 | A_96_P230769 | 2,006 | up | ---NA--- | ---NA--- |
| AK329366 | A_96_P220154 | 2,004 | up | ---NA--- | ---NA--- |
| BI924952 | A_96_P202094 | 2,002 | up | atp binding protein | C:cytoplasm |
| AK323579 | A_96_P206734 | 2,001 | up | c- sterol isomerase | F:catalytic activity; C:membrane; C:endoplasmic reticulum; P:biosynthetic process; P:cellular process; P:lipid metabolic process |
| AF179247 | A_96_P011901 | 9,315 | down | 1-aminocyclopropane-1-carboxylate synthase | P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:binding; P:ripening; F:transferase activity; F:catalytic activity |
| BF050158 | A_96_P179674 | 6,953 | down | rna-dependent rna polymerase family protein | F:RNA-directed RNA polymerase activity |
| TA36926_4081 | A_96_P090309 | 6,435 | down | protein | C:nucleus; F:hydrolase activity; P:protein metabolic process; P:catabolic process; C:membrane; C:plastid; C:thylakoid |
| AK326826 | A_96_P236823 | 5,079 | down | ---NA--- | ---NA--- |
| AK247747 | A_96_P055431 | 4,794 | down | bzip transcription factor | P:transcription; P:flower development; F:DNA binding; C:cytoplasm; F:protein binding; F:transcription factor activity; C:nucleoplasm |
| TA53803_4081 | A_96_P205009 | 4,558 | down | eix receptor 2 | F:receptor activity; F:protein binding; P:signal transduction |
| U21080 | A_96_P249177 | 4,453 | down | ---NA--- | ---NA--- |
| TA36496_4081 | A_96_P216559 | 4,229 | down | class i chitinase | F:hydrolase activity; P:carbohydrate metabolic process; P:catabolic process; C:cytoplasm; P:response to biotic stimulus; F:carbohydrate binding; P:cellular process |
| BP894496 | A_96_P216484 | 4,211 | down | aspartyl protease family protein | C:cell; P:protein metabolic process; P:catabolic process; F:hydrolase activity |
| AW649231 | A_96_P161871 | 4,116 | down | 2-oxoglutarate-dependent dioxygenase | P:biosynthetic process; P:carbohydrate metabolic process; P:cellular process; P:secondary metabolic process; F:catalytic activity |
| X71592 | A_96_P077804 | 3,968 | down | pathogenesis-related protein 1 | C:extracellular region; P:response to stress; P:response to biotic stimulus |
| AK328875 | A_96_P204784 | 3,812 | down | xenotropic and polytropic murine leukemia virus receptor ids- | P:response to extracellular stimulus; P:cell communication; P:response to stress |
| BI203353 | A_96_P196089 | 3,802 | down | copia-type pol poly | F:DNA binding; P:transcription |
| TA55370_4081 | A_96_P121272 | 3,800 | down | ---NA--- | ---NA--- |
| CK714884 | A_96_P226109 | 3,794 | down | at1g68530 t26j14_10 | P:pollination; P:multicellular organismal development; P:biosynthetic process; P:cellular process; P:lipid metabolic process; P:response to abiotic stimulus; P:response to stress; F:transferase activity; P:anatomical structure morphogenesis; P:cellular component organization; P:cell growth; C:membrane; C:endoplasmic reticulum; C:cytosol |
| BT014084 | A_96_P003801 | 3,686 | down | protein | C:plastid |

| | | | | | |
|---|---|---|---|---|---|
| DB706695 | A_96_P233494 | 3,612 | down | cytochrome p450 | F:binding; C:endoplasmic reticulum; P:metabolic process; F:molecular_function; F:catalytic activity; F:oxygen binding |
| TA47801_4081 | A_96_P252767 | 3,577 | down | | F:molecular_function; P:biological_process; C:cellular_component |
| DY523815 | A_96_P248207 | 3,416 | down | 1-aminocyclopropane-1-carboxylate oxidase | P:metabolic process; F:catalytic activity |
| TA56577_4081 | A_96_P126252 | 3,382 | down | ---NA--- | ---NA--- |
| BF097766 | A_96_P086219 | 3,235 | down | cytochrome p450 | F:binding; P:metabolic process; F:catalytic activity; F:molecular_function |
| AK327314 | A_96_P264462 | 3,213 | down | pfkb-type carbohydrate kinase family protein | F:kinase activity; P:carbohydrate metabolic process; P:cellular process; P:biological_process; C:plastid; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; P:catabolic process; P:secondary metabolic process |
| AK320058 | A_96_P227299 | 3,174 | down | protein | P:lipid metabolic process; F:triglyceride lipase activity |
| AK321656 | A_96_P170809 | 3,081 | down | protein | C:plasma membrane |
| DV103959 | A_96_P246512 | 3,074 | down | heat shock protein | F:DNA binding |
| BP908408 | A_96_P038696 | 3,059 | down | early-responsive to dehydration expressed | C:membrane; C:cytoplasm |
| AK326433 | A_96_P178449 | 3,043 | down | pectate lyase | F:catalytic activity; C:membrane |
| BI924133 | A_96_P201549 | 3,021 | down | set domain protein | F:lipid binding; F:transferase activity; P:transcription; P:flower development; P:cellular component organization; P:protein modification process; P:anatomical structure morphogenesis; C:cytoplasm; C:nucleus; C:plasma membrane |
| AK322569 | A_96_P061441 | 2,850 | down | cytochrome p450 | F:binding; F:catalytic activity |
| AK320454 | A_96_P048876 | 2,836 | down | protein | C:cell wall |
| DB710487 | A_96_P036911 | 2,835 | down | beta-galactosidase like protein | F:protein binding; F:hydrolase activity; F:carbohydrate binding; F:binding; P:carbohydrate metabolic process; P:cellular process; P:catabolic process; P:lipid metabolic process; C:cellular_component |
| AK326433 | A_96_P089729 | 2,813 | down | pectate lyase | F:catalytic activity; C:membrane |
| AK327173 | A_96_P238757 | 2,800 | down | protein | P:response to endogenous stimulus; P:biological_process; F:binding |
| AW626100 | A_96_P160701 | 2,773 | down | myb transcription factor | P:transcription; P:cellular process; P:response to endogenous stimulus; P:reproduction; P:post-embryonic development; F:transcription factor activity; C:nucleoplasm |
| BG627028 | A_96_P002531 | 2,761 | down | ---NA--- | ---NA--- |
| AK327172 | A_96_P121022 | 2,748 | down | protein | F:molecular_function; P:biological_process; C:cellular_component |
| BP906037 | A_96_P220534 | 2,735 | down | beta-glactosidase 8 | F:hydrolase activity; F:binding; F:carbohydrate binding; P:carbohydrate metabolic process; P:cellular process; P:catabolic process; P:lipid metabolic process; C:cellular_component |
| AK322569 | A_96_P203919 | 2,717 | down | cytochrome p450 | F:binding; F:catalytic activity |
| BP908452 | A_96_P221699 | 2,683 | down | cationic amino acid transporter | P:response to biotic stimulus; F:transporter activity; C:plasma membrane; P:transport; P:cellular process; C:membrane |
| AK326433 | A_96_P047806 | 2,672 | down | pectate lyase | F:catalytic activity; C:membrane |

| | | | | | |
|---|---|---|---|---|---|
| CN641307 | A_96_P226844 | 2,666 | down | protein | P:response to stress; P:response to biotic stimulus; C:plasma membrane; P:cell death; C:membrane; F:protein binding |
| BP908026 | A_96_P039016 | 2,654 | down | protein | P:biosynthetic process; P:cellular process; P:lipid metabolic process; C:plastid |
| AW979912 | A_96_P170324 | 2,654 | down | cytochrome p450 | C:cytoplasm; F:catalytic activity; F:binding; F:oxygen binding; C:endoplasmic reticulum; C:plastid |
| AK326433 | A_96_P154776 | 2,648 | down | pectate lyase | F:catalytic activity; C:membrane |
| AK329117 | A_96_P246627 | 2,644 | down | phloem protein | C:cell; F:carbohydrate binding |
| BP893421 | A_96_P216104 | 2,636 | down | protein | P:transport; P:cellular process; C:membrane; F:transporter activity |
| AW035415 | A_96_P038996 | 2,629 | down | branched-chain amino acid aminotransferase | F:transferase activity; C:plastid; C:mitochondrion; P:catabolic process; P:cellular amino acid and derivative metabolic process; P:biosynthetic process |
| AK321314 | A_96_P048831 | 2,614 | down | disease resistance-responsive family protein fibroin-related | F:binding; C:cytoplasm |
| AK319223 | A_96_P070704 | 2,601 | down | mudrA protein-maize transposon MuDR | F:nucleic acid binding |
| AK247863 | A_96_P237947 | 2,598 | down | protein | F:binding |
| AI782347 | A_96_P138892 | 2,596 | down | zinc finger (b-box type) family protein | P:transcription; C:plasma membrane; F:protein binding; F:transcription factor activity; F:binding; C:nucleoplasm |
| AK324806 | A_96_P233139 | 2,559 | down | nodulin 21 family protein | C:cell |
| AW623437 | A_96_P159071 | 2,539 | down | inosine-uridine preferring nucleoside | C:cytoplasm; F:hydrolase activity; C:cell wall; C:mitochondrion |
| AK323186 | A_96_P230709 | 2,506 | down | protein | C:plastid; F:molecular_function; P:biological_process |
| BM411716 | A_96_P208209 | 2,504 | down | pectate lyase | F:catalytic activity; C:membrane |
| X98929 | A_96_P000061 | 2,494 | down | subtilisin-like protease | F:protein binding; C:cell wall; F:hydrolase activity; C:cytoplasm; P:reproduction; P:post-embryonic development; P:metabolic process; P:cellular process; P:protein metabolic process; P:catabolic process; P:biological_process; C:extracellular region |
| X98929 | A_96_P110762 | 2,471 | down | subtilisin-like protease | F:protein binding; C:cell wall; F:hydrolase activity; C:cytoplasm; P:reproduction; P:post-embryonic development; P:metabolic process; P:cellular process; P:protein metabolic process; P:catabolic process; P:biological_process; C:extracellular region |
| TA56692_4081 | A_96_P126712 | 2,467 | down | heat shock protein binding | P:protein metabolic process; P:cellular process |
| AK325623 | A_96_P234029 | 2,457 | down | protein | C:cell; C:cytoplasm; C:vacuole; C:cell wall |
| AW036000 | A_96_P030741 | 2,452 | down | sulfate bicarbonate oxalate exchanger and transporter sat-1 | C:membrane; F:transporter activity; P:transport; P:cellular process |
| TA52817_4081 | A_96_P111377 | 2,432 | down | n-acylglucosamine 2-epimerase | P:carbohydrate metabolic process; P:cellular process; F:catalytic activity |

| | | | | | |
|---|---|---|---|---|---|
| AK320138 | A_96_P031762 | 2,431 | down | glycosyl group 1 family protein | F:transferase activity; P:biosynthetic process; P:cellular process; P:lipid metabolic process; P:response to extracellular stimulus; P:cell communication; P:response to stress; C:plastid |
| AF179249 | A_96_P095644 | 2,425 | down | 1-aminocyclopropane-1-carboxylate synthase | F:transferase activity; F:binding; P:biosynthetic process; F:catalytic activity; P:cellular amino acid and derivative metabolic process |
| AK326433 | A_96_P089719 | 2,408 | down | pectate lyase | F:catalytic activity; C:membrane |
| BF097299 | A_96_P181749 | 2,402 | down | protein | C:cytoplasm; F:hydrolase activity; P:protein metabolic process; P:catabolic process; C:extracellular region |
| DB718737 | A_96_P209849 | 2,396 | down | protein | F:catalytic activity |
| BG130811 | A_96_P026736 | 2,385 | down | phosphoenolpyruvate carboxylase | C:cytoplasm; P:metabolic process; P:generation of precursor metabolites and energy; P:catabolic process; P:photosynthesis; F:catalytic activity; P:biosynthetic process; P:carbohydrate metabolic process; P:cellular process |
| DB716608 | A_96_P067916 | 2,381 | down | sterol regulatory element-binding protein site 2 | F:hydrolase activity; P:response to abiotic stimulus; P:response to stress; C:membrane; C:plastid |
| AK319877 | A_96_P220429 | 2,371 | down | ferric reductase-like transmembrane component | F:catalytic activity; C:plasma membrane; F:molecular_function; F:binding; P:generation of precursor metabolites and energy; P:photosynthesis; C:membrane; F:nucleotide binding; C:plastid; P:response to abiotic stimulus |
| BG130117 | A_96_P064386 | 2,360 | down | swim zinc finger family protein | F:binding |
| AK328502 | A_96_P229454 | 2,359 | down | bim1 dna binding protein binding transcription factor | F:protein binding; F:transcription factor activity; P:response to endogenous stimulus; P:signal transduction; C:nucleoplasm; P:transcription |
| AI777995 | A_96_P033561 | 2,356 | down | protein | P:biosynthetic process; P:cellular process; P:lipid metabolic process |
| DB683870 | A_96_P174494 | 2,345 | down | serine acetyltransferase | C:cytosol; F:binding; C:mitochondrion; P:response to stress; P:response to abiotic stimulus; F:transferase activity; P:response to extracellular stimulus; P:cell communication; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; C:plastid |
| AK324190 | A_96_P232399 | 2,344 | down | glutamate-gated kainate-type ion channel receptor subunit 5 | F:transporter activity; P:transport; F:receptor activity; C:membrane; C:external encapsulating structure; P:cell-cell signaling; P:signal transduction |
| BG127331 | A_96_P033651 | 2,332 | down | gh3-like protein | P:response to endogenous stimulus; P:response to abiotic stimulus |
| AK319877 | A_96_P093054 | 2,330 | down | ferric reductase-like transmembrane component | F:catalytic activity; C:plasma membrane; F:molecular_function; F:binding; P:generation of precursor metabolites and energy; P:photosynthesis; C:membrane; F:nucleotide binding; C:plastid; P:response to abiotic stimulus |
| U13055 | A_96_P245140 | 2,329 | down | endo- -beta-glucanase precursor | F:hydrolase activity; P:cellular component organization; P:cell growth; P:abscission; P:cellular process; P:carbohydrate metabolic process |
| AK325209 | A_96_P064151 | 2,328 | down | constans 1 | F:binding; C:intracellular |
| AK324830 | A_96_P246702 | 2,318 | down | beta-glucosidase 47 | P:carbohydrate metabolic process; F:hydrolase activity; F:binding; C:cytoplasm; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| AW738136 | A_96_P164401 | 2,309 | down | adaptin family protein | C:Golgi apparatus; F:protein binding; C:membrane; C:cytoplasm; F:transporter activity; P:transport; P:cellular process; P:cellular component organization |

| | | | | | |
|---|---|---|---|---|---|
| BM410928 | A_96_P043161 | 2,308 | down | nac domain ipr003441 | P:biological_process; P:response to stress; P:transcription; F:transcription factor activity; F:protein binding; C:nucleoplasm |
| AK326433 | A_96_P173504 | 2,302 | down | pectate lyase | F:catalytic activity; C:membrane |
| AI778144 | A_96_P136922 | 2,297 | down | beta-cyanoalanine synthase | F:binding; F:catalytic activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:transferase activity; C:cytoplasm |
| AK326433 | A_96_P155111 | 2,291 | down | pectate lyase | F:catalytic activity; C:membrane |
| AK320732 | A_96_P184239 | 2,281 | down | chitinase 134 | F:hydrolase activity; C:extracellular space; P:carbohydrate metabolic process; P:catabolic process; P:response to stress; F:carbohydrate binding; P:cellular process |
| AK323358 | A_96_P003831 | 2,277 | down | protein | C:plasma membrane |
| AI777546 | A_96_P136642 | 2,251 | down | gh3-like protein | P:response to endogenous stimulus; P:response to abiotic stimulus |
| AK327977 | A_96_P218029 | 2,247 | down | cbs domain-containing protein | P:response to stress; P:response to abiotic stimulus; C:mitochondrion; F:binding |
| AK320322 | A_96_P227554 | 2,245 | down | malate synthase | F:transferase activity; P:generation of precursor metabolites and energy; P:catabolic process; C:peroxisome; F:catalytic activity; P:carbohydrate metabolic process; P:cellular process; P:biosynthetic process; P:metabolic process |
| AK324526 | A_96_P042041 | 2,219 | down | protein | P:carbohydrate metabolic process; C:extracellular region; F:hydrolase activity; P:reproduction; P:post-embryonic development; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process |
| AK325889 | A_96_P171069 | 2,218 | down | protein | P:metabolic process; P:cellular process; F:binding; P:response to extracellular stimulus; P:cell communication; P:response to stress |
| BG134199 | A_96_P190219 | 2,214 | down | major intrinsic protein | P:transport; P:cellular process; C:membrane; F:transporter activity |
| AF154003 | A_96_P012826 | 2,202 | down | pirin-like protein | C:nucleus; C:mitochondrion; C:plastid |
| BG135790 | A_96_P191179 | 2,193 | down | dicer-like protein | F:nuclease activity; F:RNA binding; P:regulation of gene expression, epigenetic; P:DNA metabolic process; C:intracellular; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; F:hydrolase activity; P:response to stress; P:response to biotic stimulus |
| AK247754 | A_96_P103539 | 2,190 | down | ---NA--- | ---NA--- |
| BW689058 | A_96_P223779 | 2,188 | down | ---NA--- | ---NA--- |
| AK320574 | A_96_P058886 | 2,186 | down | high affinity nitrate transporter | C:membrane; C:vacuole; P:transport; P:cellular process; F:transporter activity |
| AK327201 | A_96_P238928 | 2,182 | down | ---NA--- | ---NA--- |
| AK247493 | A_96_P171789 | 2,175 | down | leucine-rich repeat family protein | P:biological_process; F:catalytic activity; C:cytoplasm; C:plasma membrane; P:response to biotic stimulus; F:protein binding; P:signal transduction; C:plastid |
| BT013433 | A_96_P259447 | 2,173 | down | inducer of cbf expression 1 | P:post-embryonic development; P:cellular process; F:DNA binding; F:transcription regulator activity; P:response to stress; P:response to abiotic stimulus; C:nucleus; P:transcription |
| BI927023 | A_96_P027451 | 2,172 | down | chalcone synthase family protein | F:transferase activity; P:anatomical structure morphogenesis; P:cellular component organization; P:multicellular organismal development; P:cellular process; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |

| | | | | | |
|---|---|---|---|---|---|
| BI208039 | A_96_P197839 | 2,169 | down | ---NA--- | ---NA--- |
| AK323674 | A_96_P231489 | 2,165 | down | delta-12 oleate desaturase | P:metabolic process; F:catalytic activity; P:lipid metabolic process |
| BT014116 | A_96_P222569 | 2,155 | down | acr3 amino acid binding | P:metabolic process; C:cytosol; F:binding |
| AK324231 | A_96_P052956 | 2,150 | down | lipid binding | C:cytoplasm; C:mitochondrion |
| AB004558 | A_96_P011826 | 2,146 | down | cell-wall invertase | C:extracellular region; F:hydrolase activity; C:cell wall; P:carbohydrate metabolic process; P:cellular process |
| AK321479 | A_96_P163046 | 2,143 | down | cytochrome p450 | F:binding; P:metabolic process; F:catalytic activity; F:molecular_function |
| AK322310 | A_96_P103969 | 2,122 | down | glucosyl transferase | F:transferase activity; P:metabolic process |
| AK324313 | A_96_P056716 | 2,119 | down | beta-cyanoalanine synthase | F:binding; P:metabolic process; P:response to stress; C:mitochondrion; P:cellular process; F:transferase activity; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity; C:cytoplasm |
| AW615915 | A_96_P157571 | 2,116 | down | potyviral helper component protease-interacting protein 1 | F:binding; F:protein binding; F:hydrolase activity |
| BT012876 | A_96_P254477 | 2,115 | down | protein | C:membrane; C:Golgi apparatus; F:protein binding; P:transport; P:cellular process; F:binding; F:transporter activity |
| TA36796_4081 | A_96_P089969 | 2,110 | down | protein | F:transferase activity; P:cellular component organization; P:response to stress; P:cellular process; P:response to endogenous stimulus; P:response to biotic stimulus; P:response to abiotic stimulus; P:biosynthetic process; P:carbohydrate metabolic process |
| AK328261 | A_96_P068741 | 2,109 | down | gibberellin 2-oxidase | P:metabolic process; F:binding; F:catalytic activity; P:biosynthetic process; P:cellular process; P:lipid metabolic process; P:secondary metabolic process |
| AK324830 | A_96_P233169 | 2,107 | down | beta-glucosidase 47 | P:carbohydrate metabolic process; F:hydrolase activity; F:binding; C:cytoplasm; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; P:secondary metabolic process |
| AF096776 | A_96_P012926 | 2,106 | down | expansin | C:extracellular region; P:cellular component organization; P:cellular process; C:membrane |
| AK247493 | A_96_P107949 | 2,105 | down | leucine-rich repeat family protein | P:biological_process; F:catalytic activity; C:cytoplasm; C:plasma membrane; P:response to biotic stimulus; F:protein binding; P:signal transduction; C:plastid |
| TA36711_4081 | A_96_P178714 | 2,097 | down | pectate lyase | F:catalytic activity |
| DB689894 | A_96_P229714 | 2,096 | down | kelch repeat-containing f-box family | F:protein binding |
| BF098346 | A_96_P182694 | 2,091 | down | pho1-like protein | P:transport; P:response to extracellular stimulus; P:cell communication; P:response to stress |
| BG627885 | A_96_P003171 | 2,087 | down | ---NA--- | ---NA--- |
| DB724515 | A_96_P059476 | 2,084 | down | nucleotide binding protein | C:cytosol; F:binding; F:nucleotide binding; F:protein binding |
| TA47182_4081 | A_96_P260397 | 2,081 | down | cyclic nucleotide-gated ion | P:transport; P:cellular process; F:transporter activity; F:protein binding; C:plasma membrane; C:intracellular; C:membrane |

| | | | | | |
|---|---|---|---|---|---|
| TA45765_4081 | A_96_P162791 | 2,074 | down | regulator of chromosome condensation family protein | F:binding |
| AW219022 | A_96_P046311 | 2,073 | down | mads-box protein | P:response to endogenous stimulus; P:transport; P:cellular process; P:transcription; P:response to stress; P:response to abiotic stimulus; P:flower development; F:DNA binding; F:transcription regulator activity; F:protein binding; F:transcription factor activity; C:cytoplasm; C:nucleoplasm |
| TA37481_4081 | A_96_P172969 | 2,073 | down | 4-coumarate- ligase-like protein | F:nucleotide binding; P:biological_process; C:plastid; C:extracellular region; F:catalytic activity; P:biosynthetic process; P:cellular process |
| BP897166 | A_96_P259463 | 2,072 | down | ribulose bisphosphate carboxylase | P:biosynthetic process; P:carbohydrate metabolic process; P:photosynthesis; F:catalytic activity; P:cellular process; F:protein binding; P:metabolic process; C:plastid |
| AK323674 | A_96_P090844 | 2,064 | down | delta-12 oleate desaturase | P:metabolic process; F:catalytic activity; P:lipid metabolic process |
| AJ319999 | A_96_P083809 | 2,061 | down | ---NA--- | ---NA--- |
| TA56280_4081 | A_96_P125022 | 2,051 | down | hexose transporter | C:cell; P:transport; P:cellular process; F:transporter activity; P:metabolic process; C:membrane; F:catalytic activity |
| L24012 | A_96_P014036 | 2,051 | down | aminotransferase | F:transferase activity |
| BG133784 | A_96_P189949 | 2,046 | down | protein | F:kinase activity |
| TA36295_4081 | A_96_P088464 | 2,042 | down | glyceraldehyde-3-phosphate dehydrogenase | P:metabolic process; F:catalytic activity; F:nucleotide binding; P:biosynthetic process; P:carbohydrate metabolic process; P:cellular process; P:generation of precursor metabolites and energy; P:catabolic process |
| AK327122 | A_96_P017621 | 2,041 | down | regulator of chromosome condensation family protein | F:kinase activity; F:chromatin binding; F:protein binding; C:intracellular |
| TA39043_4081 | A_96_P094529 | 2,037 | down | serine acetyltransferase | C:cytosol; F:transcription regulator activity; F:binding; C:mitochondrion; P:response to stress; P:response to abiotic stimulus; P:transcription; F:transferase activity; P:response to extracellular stimulus; P:cell communication; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; C:plastid; C:nucleus |
| BE450553 | A_96_P044361 | 2,034 | down | dna binding | F:DNA binding |
| BM411846 | A_96_P042286 | 2,030 | down | microtubule-associated protein | P:cellular process; P:response to stress; P:response to abiotic stimulus; P:biological_process; P:response to endogenous stimulus; P:cellular component organization; P:signal transduction; F:protein binding; C:cytoskeleton |
| BG123415 | A_96_P184454 | 2,025 | down | fructose- -bisphosphatase | F:binding; P:metabolic process; C:cytoplasm; F:hydrolase activity; F:catalytic activity; P:carbohydrate metabolic process; P:cellular process; P:biosynthetic process; P:generation of precursor metabolites and energy; P:catabolic process; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; P:secondary metabolic process |
| AF179249 | A_96_P095641 | 2,024 | down | 1-aminocyclopropane-1-carboxylate synthase | F:transferase activity; F:binding; P:biosynthetic process; F:catalytic activity; P:cellular amino acid and derivative metabolic process |
| AK327992 | A_96_P230894 | 2,023 | down | mads box | P:multicellular organismal development; F:DNA binding; F:transcription factor activity; P:transcription; C:nucleoplasm |

| | | | | | |
|---|---|---|---|---|---|
| AK328892 | A_96_P055806 | 2,023 | down | protein | C:cytoplasm; F:receptor activity; F:protein binding; C:nucleus; P:response to endogenous stimulus; P:signal transduction; F:lipid binding |
| AK320322 | A_96_P027151 | 2,023 | down | malate synthase | F:transferase activity; P:generation of precursor metabolites and energy; P:catabolic process; C:peroxisome; F:catalytic activity; P:carbohydrate metabolic process; P:cellular process; P:biosynthetic process; P:metabolic process |
| AK326775 | A_96_P092354 | 2,022 | down | aldehyde dehydrogenase | F:catalytic activity; P:biological_process; P:metabolic process; F:nucleotide binding; C:mitochondrion; P:biosynthetic process; P:carbohydrate metabolic process; P:cellular process; P:generation of precursor metabolites and energy; P:catabolic process; P:cellular amino acid and derivative metabolic process; P:lipid metabolic process; P:secondary metabolic process |
| AW625874 | A_96_P160571 | 2,021 | down | arabinogalactan protein | C:cytosol; C:mitochondrion; C:plasma membrane |
| DB681453 | A_96_P058301 | 2,019 | down | nodulin family protein | C:cell |
| BI932283 | A_96_P205759 | 2,019 | down | ---NA--- | ---NA--- |
| TA56993_4081 | A_96_P127927 | 2,018 | down | protein | F:binding |
| BP891117 | A_96_P215284 | 2,015 | down | ---NA--- | ---NA--- |
| AK325814 | A_96_P063191 | 2,013 | down | protein | F:receptor activity; F:protein binding; P:catabolic process; P:cellular process; P:signal transduction; C:cytoskeleton |
| AK329166 | A_96_P110417 | 2,006 | down | conserved hypothetical protein [Ricinus communis] | C:cytoplasm |
| M80608 | A_96_P143586 | 2,004 | down | Err:509 | P:response to stress; C:vacuole; F:hydrolase activity; F:binding; P:carbohydrate metabolic process; P:cellular process |
| DB727290 | A_96_P246190 | 2,002 | down | aaa-type atpase family protein | C:mitochondrion; F:nucleotide binding |
| TA48673_4081 | A_96_P106864 | 2,001 | down | basic 7s globulin 2 precursor small | F:structural molecule activity; C:thylakoid; C:plastid; P:biological_process |

**Table A2. List of 309 Arabidopsis proteins corresponding to RSYS differentially expressed sequences. RSYS network includes 195 proteins underlined with grey colour.**

| Tom ESTs | Probe Name | FC | Reg | Description | GOs | RefSeq ID | TAIR ID | Gene Symbol | Arabidopsis protein |
|---|---|---|---|---|---|---|---|---|---|
| AF083253 | A_96_P016051 | 211,72 | up | cysteine proteinase inhibitor | F:hydrolase activity; F:enzyme regulator activity | NP_566425 | AT3G12490 | ATCYS6 | cystatin B |
| AI488671 | A_96_P132022 | 94,57 | up | proteinase inhibitor i | F:enzyme regulator activity | NP_030435 | AT2G38870 | | serine protease inhibitor, potato inhibitor I-type protein |
| U50152 | A_96_P000336 | 82,20 | up | leucine aminopeptidase | C:vacuole; F:binding; F:hydrolase activity;P:response to stress; | NP_194821 | AT4G30920 | AT4G30920 | Cytosol aminopeptidase family protein |
| AY534531 | A_96_P173409 | 71,32 | up | alcohol acyl transferase | F:transferase activity; P:biosynthetic process | NP_197256 | AT5G17540 | | HXXXD-type acyl-transferase-like protein |
| AW738222 | A_96_P011621 | 49,62 | up | trypsin and protease inhibitor family protein | F:enzyme regulator activity; F:hydrolase activity | NP_565062 | AT1G73325 | | Kunitz family trypsin and protease inhibitor protein |
| GO372786 | A_96_P011246 | 22,52 | up | endoribonuclease l-psp family protein | C:mitochondrion; C:plastid; F:nuclease activity; | NP_188674 | AT3G20390 | AT3G20390 | endoribonuclease L-PSP family protein |
| M61914 | A_96_P205969 | 17,88 | up | threonine deaminase | P:cellular amino acid and derivative metabolic process; P:response to stress; C:plastid | NP_187616 | AT3G10050 | OMR1 | OMR1__L-O-methylthreonine resistant 1 |
| AK321112 | A_96_P011291 | 17,50 | up | arginase | C:mitochondrion; P:response to biotic stimulus; F:hydrolase activity; C:plastid | NP_192629 | AT4G08900 | AT4G08900 | arginase |
| ES897217 | A_96_P171804 | 15,53 | up | kunitz-type protease inhibitor precursor | C:vacuole; P:response to stress; F:hydrolase activity | NP_173228 | AT1G17860 | | kunitz type trypsin and protease inhibitor domain-containing protein |
| AK323732 | A_96_P010533 | 13,57 | up | stem 28 kda glycoprotein | C:cytoplasm; F:hydrolase activity | NP_194245 | AT4G25150 | AT4G25150 | HAD superfamily |
| BI931087 | A_96_P040851 | 13,00 | up | myb-related transcription factor | P:transcription;C:nucleus; P:response to stress; P:response to abiotic stimulus | NP_172108 | AT1G06180 | | myb proto-oncogene protein |
| AK320125 | A_96_P071899 | 12,94 | up | anthranilate n-hydroxycinnamoyl benzoyltransferase | F:transferase activity | NP_201516 | AT5G67150 | AT5G67150 | transferase family protein |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AK320918 | A_96_P197769 | 10,80 | up | protein | P:cellular amino acid and derivative metabolic process; P:secondary metabolic process | NP_196179 | AT5G05600 | AT5G05600 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein |
| TA38526_4081 | A_96_P093601 | 9,55 | up | subtilisin-like protease | P:protein metabolic process; F:protein binding; F:hydrolase activity | NP_568765 | AT5G51750 | | subtilase 1.3 |
| AW648744 | A_96_P054411 | 9,54 | up | iaa-amino acid hydrolase ilr1 | F:protein binding; F:hydrolase activity; | NP_175587 | AT1G51760 | IAR3 | peptidase M20/M25/M40 family protein |
| AK328079 | A_96_P011176 | 8,83 | up | peptidase m20 m25 m40 family protein | F:hydrolase activity; P:protein metabolic process; | NP_193517 | AT4G17830 | AT4G17830 | Peptidase M20/M25/M40 family protein |
| AJ271093 | A_96_P000051 | 8,76 | up | allene oxide synthase | F:catalytic activity; F:binding; F:molecular_function | NP_199079 | AT5G42650 | | allene oxide synthase |
| BI924537 | A_96_P201884 | 8,63 | up | purple acid phosphatase | C:cytoplasm; F:binding; F:hydrolase activity; F:protein binding; P:protein modification process; | NP_199851 | AT5G50400 | | purple acid phosphatase 27 |
| AK329477 | A_96_P042771 | 7,25 | up | pr-10 type pathogenesis-related protein | P:response to biotic stimulus; P:response to stress | NP_177245 | AT1G70890 | | MLP-like protein 43 |
| EG553806 | A_96_P054926 | 7,13 | up | protein | F:molecular_function; P:biological_process; C:cellular_component | NP_567264 | AT4G04630 | | uncharacterized protein |
| AW429114 | A_96_P155641 | 6,49 | up | ac091627_9amidase family protein | C:cytoplasm; F:catalytic activity | NP_195214 | AT4G34880 | AT4G34880 | Amidase family protein |
| BI931662 | A_96_P021601 | 6,10 | up | auxin efflux carrier family protein | P:transport; C:cell; C:membrane; F:transporter activity; P:cellular process | NP_683316 | AT1G20925 | AT1G20925 | auxin:hydrogen symporter activity |
| BI206321 | A_96_P023961 | 5,79 | up | calcium-dependent protein kinase | F:kinase activity; P:protein modification process; | NP_201422 | AT5G66210 | CPK28 | CPK28__calcium-dependent protein kinase 28 |
| AI486264 | A_96_P257717 | 5,75 | up | anthocyanin acyltransferase | F:transferase activity | NP_173852 | AT1G24430 | | HXXXD-type acyl-transferase-like protein |
| AK322858 | A_96_P100214 | 5,62 | up | 3-ketoacyl- thiolase | P:cellular component organization;C:peroxisome; C:mitochondrion; C:nucleolus; F:transferase activity; P:response to stress; C:plastid; C:plasma membrane; | NP_180873 | AT2G33150 | | 3-ketoacyl-CoA thiolase 2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BG129343 | A_96_P262272 | 5,55 | up | anthocyanin acyltransferase | F:transferase activity; P:biological_process | NP_173851 | AT1G24420 | AT1G24420 | HXXXD-type acyl-transferase family protein |
| AY840091 | A_96_P001996 | 5,37 | up | (-)-a-terpineol synthase | P:lipid metabolic process; P:secondary metabolic process; F:binding; F:catalytic activity | NP_189209 | AT3G25810 | AT3G25810 | Terpenoid cyclases/Protein prenyltransferases superfamily protein |
| BI207729 | A_96_P033061 | 5,36 | up | jasmonate zim-domain protein 1 | P:signal transduction; P:response to stress; P:response to biotic stimulus | NP_565096 | AT1G74950 | TIFY10 | TIFY domain/Divergent CCT motif family protein |
| AW035871 | A_96_P146991 | 5,34 | up | lactoylglutathione lyase | F:catalytic activity | NP_565231 | AT1G80160 | | lactoylglutathione lyase / glyoxalase I-like protein |
| AK325215 | A_96_P018261 | 5,31 | up | beta-alanine-pyruvate aminotransferase | P:cell communication; C:mitochondrion; | NP_187498 | AT3G08860 | PYD4 | PYRIMIDINE 4. |
| TA54114_ 4081 | A_96_P116022 | 5,27 | up | MYB transcription factor | P:response to stress; P:response to abiotic stimulus; P:transcription; C:nucleoplasm | NP_564176 | AT1G22640 | | transcription factor MYB3 |
| AK323178 | A_96_P130852 | 5,27 | up | protein | C:vacuole; C:cell wall; F:hydrolase activity; P:catabolic process; P:lipid metabolic process | NP_174181 | AT1G28590 | AT1G28590 | GDSL-like Lipase/Acylhydrolase superfamily protein. |
| TA43917_ 4081 | A_96_P141222 | 5,24 | up | xanthine dehydrogenase | P:metabolic process; F:catalytic activity; P:response to stress; F:nucleotide binding | NP_195215 | AT4G34890 | | xanthine dehydrogenase 1 |
| AK327683 | A_96_P248562 | 4,98 | up | jasmonate zim-domain protein 1 | P:response to endogenous stimulus;P:response to stress | NP_564075 | AT1G19180 | JAZ1 | JAZIM1_ jasmonic acid mediated signaling pathway |
| AK319704 | A_96_P094299 | 4,95 | up | anthocyanin acyltransferase | F:transferase activity; P:biological_process | NP_189233 | AT3G26040 | | HXXXD-type acyl-transferase-like protein |
| ES894405 | A_96_P191629 | 4,74 | up | s-adenosyl-l-methionine | F:transferase activity | NP_173394 | AT1G19640 | | jasmonic acid carboxyl methyltransferase |
| BP878428 | A_96_P210104 | 4,50 | up | subtilisin-like protease | F:hydrolase activity | NP_568896 | AT5G59100 | | Subtilisin-like serine endopeptidase family protein |
| BI206363 | A_96_P197229 | 4,34 | up | mate efflux family protein | P:response to biotic stimulus; C:vacuole; F:transporter activity; C:plasma membrane | NP_189291 | AT3G26590 | AT3G26590 | MATE efflux family protein |
| AI898504 | A_96_P141022 | 4,34 | up | ac007354_12 gb | C:cell; C:cytoplasm | NP_973806 | AT1G10740 | | alpha/beta-hydrolase domain-containing protein |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BI924804 | A_96_P202044 | 4,24 | up | spermidine synthase | C:cytoplasm; F:transferase activity; P:secondary metabolic process | NP_568785 | AT5G53120 | ATSPDS3 | ATSPDS3_SPDS3_SPMS__spermidine synthase 3 |
| AK324101 | A_96_P002246 | 4,17 | up | lipid-associated family protein | C:vacuole; C:membrane; C:plastid; C:thylakoid; C:plasma membrane | NP_565527 | AT2G22170 | | PLAT-plant-stress domain-containing protein |
| AK325249 | A_96_P126582 | 3,98 | up | amino acid transporter | C:membrane; P:transport; F:transporter activity | NP_197028 | AT5G15240 | AT5G15240 | Transmembrane amino acid transporter family protein |
| AK321920 | A_96_P225949 | 3,86 | up | protein | F:receptor activity; C:membrane; F:catalytic activity; P:signal transduction | NP_201331 | AT5G65280 | GCL1 | GCL1__GCR2-like 1 |
| AK319887 | A_96_P193799 | 3,82 | up | mta sah | F:binding; C:cytoplasm; P:protein modification process; C:intracellular | NP_194623 | AT4G28940 | | Phosphorylase-like protein protein |
| U37840 | A_96_P151446 | 3,73 | up | lipoxygenase | P:response to biotic stimulus; P:growth; C:plastid; P:response to abiotic stimulus; F:binding | NP_564021 | AT1G17420 | | lipoxygenase 3 |
| AK324775 | A_96_P121307 | 3,70 | up | serine carboxypeptidase 1 precursor-like protein | C:extracellular region; C:peroxisome; F:hydrolase activity; | NP_193027 | AT4G12910 | scpl20 | scpl20__serine carboxypeptidase-like 20 |
| BI933291 | A_96_P038801 | 3,68 | up | phenylalanine ammonia-lyase | C:cytoplasm; P:secondary metabolic process; P:catabolic process | NP_187645 | AT3G10340 | PAL4 | phenylalanine ammonia-lyase 4 |
| AJ831864 | A_96_P004791 | 3,66 | up | non-specific lipid transfer protein | P:response to stress; F:lipid binding; P:transport | NP_181388 | AT2G38540 | LP1 | lipid transfer protein 1. |
| TA50399_4081 | A_96_P108637 | 3,61 | up | mate efflux family protein | P:response to biotic stimulus; C:vacuole; F:transporter activity; C:plasma membrane; | NP_172755 | AT1G12950 | | root hair specific 2 |
| AW929506 | A_96_P027301 | 3,46 | up | phenylalanine ammonia-lyase | C:cytoplasm; P:secondary metabolic process; P:catabolic process; P:cellular process | NP_181241 | AT2G37040 | | phenylalanine ammonia-lyase 1 |
| BI929069 | A_96_P204479 | 3,31 | up | white-brown-complex abc transporter family | F:nucleotide binding; F:hydrolase activity; F:transporter activity; C:membrane; P:transport | NP_173226 | AT1G17840 | WBC11 | ABCG11_ATWBC11_COF1_DSO_WBC11__white-brown complex homolog protein 11 |
| TA36168_4081 | A_96_P088096 | 3,27 | up | 24-sterol c-methyltransferase | P:cell cycle; P:cell growth; C:endoplasmic reticulum; F:transferase activity; | NP_173458 | AT1G20330 | | 24-methylenesterol C-methyltransferase 2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| DQ399832 | A_96_P068711 | 3,25 | up | gras family transcription factor | P:transcription; P:biological_process; F:transcription factor activity; C:nucleoplasm | NP_188000 | AT3G13840 | | scarecrow-like protein 29 |
| X04792 | A_96_P074929 | 3,23 | up | 1-aminocyclopropane-1-carboxylate oxidase | F:binding; P:biosynthetic process; P:response to stress; P:ripening; | NP_171994 | AT1G05010 | EFE | ethylene-forming enzyme. |
| AK324373 | A_96_P232679 | 3,23 | up | calmodulin | P:response to external stimulus; P:response to abiotic stimulus; F:signal transducer activity; | NP_176814 | AT1G66410 | CAM4 | ACAM-4_CAM4__calmodulin 4 |
| AK322239 | A_96_P072419 | 3,18 | up | caffeoyl- o-methyltransferase | F:transferase activity; F:nucleic acid binding; C:cytosol; P:secondary metabolic process | NP_567739 | AT4G26220 | AT4G26220 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein |
| AK326904 | A_96_P048996 | 3,14 | up | fumarylacetoacetate hydrolase | F:hydrolase activity; P:catabolic process; P:cellular process | NP_172669 | AT1G12050 | AT1G12050 | fumarylacetoacetase |
| BP895928 | A_96_P106199 | 3,11 | up | nucleotidyltransferase domain containing expressed | P:transcription; F:transferase activity | NP_191728 | AT3G61690 | | nucleotidyltransferase |
| U20592 | A_96_P258022 | 2,99 | up | kunitz-type protease inhibitor kpi- | F:enzyme regulator activity | NP_565061 | AT1G73260 | | kunitz trypsin inhibitor 1 |
| AK328455 | A_96_P047426 | 2,93 | up | protein | F:binding; F:transferase activity; P:catabolic process; C:cytosol | NP_176647 | AT1G64660 | | methionine gamma-lyase |
| AK328900 | A_96_P152676 | 2,93 | up | tropinone reductase | P:metabolic process; P:biological_process; F:binding; F:catalytic activity | NP_196225 | AT5G06060 | AT5G06060 | NAD(P)-binding Rossmann-fold superfamily protein |
| BG126724 | A_96_P185909 | 2,92 | up | MYB transcription factor | F:DNA binding; P:transcription; C:nucleus | NP_190575 | AT3G50060 | MYB77 | MYB77__myb domain protein 77 |
| AK322540 | A_96_P092624 | 2,88 | up | 3-beta hydroxysteroid dehydrogenase | F:binding; P:metabolic process; F:catalytic activity | NP_566097 | AT2G47140 | AT2G47140 | NAD(P)-binding Rossmann-fold superfamily protein |
| AK324283 | A_96_P217814 | 2,87 | up | chlorophyllase 2 | P:response to stress; C:vacuole; F:hydrolase activity; P:catabolic process | NP_199199 | AT5G43860 | | chlorophyllase 2 |
| AK325661 | A_96_P063166 | 2,86 | up | retroelement pol polyprotein | F:nuclease activity; F:DNA binding; P:transport; P:signal transduction; | NP_194047 | AT4G23160 | | cysteine-rich receptor-like protein kinase 8 |
| AK320183 | A_96_P232574 | 2,79 | up | homogentisate - dioxygenase | P:catabolic process; F:catalytic activity; P:metabolic | NP_200219 | AT5G54080 | HGO | HGO__homogentisate 1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | process; P:cellular process | | | | |
| AK328438 | A_96_P189614 | 2,77 | up | 4-coumarate: ligase | F:nucleotide binding; F:catalytic activity; P:secondary metabolic process | NP_175579 | AT1G51680 | 4CL.1 | 4CL.1_4CL1_AT4CL1__4-coumarate:CoA ligase 1 |
| EG553122 | A_96_P031791 | 2,76 | up | ap2 erf domain-containing transcription factor | P:biological_process; F:DNA binding; P:transcription | NP_195167 | AT4G34410 | RRTF1 | RRTF1__redox responsive transcription factor 1 |
| BG126168 | A_96_P185699 | 2,76 | up | ap2 domain-containing transcription factor | P:anatomical structure morphogenesis;F:transcription factor activity; C:nucleoplasm | NP_565674 | AT2G28550 | TOE1 | RAP2.7_TOE1__related to AP2.7 |
| AK324054 | A_96_P198409 | 2,74 | up | glucan endo- -beta-glucosidase | C:cell wall; C:plasma membrane; C:cytoplasm; P:carbohydrate metabolic process; | NP_849556 | AT4G29360 | AT4G29360 | O-Glycosyl hydrolases family 17 protein |
| AK322920 | A_96_P095579 | 2,73 | up | protein | C:membrane | NP_849772 | AT1G45201 | | triacylglycerol lipase-like 1 protein |
| AK323867 | A_96_P081974 | 2,72 | up | annexin 11 | P:response to endogenous stimulus; P:response to stress; P:response to abiotic stimulus; | NP_181409 | AT2G38750 | ANNAT4 | ANNAT4__annexin 4 |
| BW689995 | A_96_P052621 | 2,71 | up | translocon-associated protein beta family protein | C:cytoplasm; C:plasma membrane | NP_568293 | AT5G14030 | AT5G14030 | translocon-associated protein beta (TRAPB) family protein |
| DB711714 | A_96_P068811 | 2,69 | up | myosin | P:transport; P:cellular process; F:motor activity; C:cytoskeleton | NP_179917 | AT2G23360 | | filament-like plant protein 7 |
| AW625803 | A_96_P030801 | 2,68 | up | hydroxycinnamoyltransferase | F:transferase activity; P:biosynthetic process | NP_199704 | AT5G48930 | | hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase |
| AK320848 | A_96_P004291 | 2,68 | up | Err:509 | P:metabolic process; F:binding; P:cellular process; F:catalytic activity | NP_567456 | AT4G15093 | AT4G15093 | catalytic LigB subunit of aromatic ring-opening dioxygenase family |
| AK321533 | A_96_P048951 | 2,66 | up | protein | C:membrane | NP_564374 | AT1G31130 | AT1G31130 | unknown protein |
| BI922494 | A_96_P200799 | 2,66 | up | nucleic acid binding nucleotide binding | F:binding | NP_001118504 | AT2G42240 | | RNA recognition motif-containing protein |
| DB704225 | A_96_P034902 | 2,65 | up | glutathione s-transferase | F:transferase activity | NP_195899 | AT5G02790 | GSTL3 | GSTL3__Glutathione S-transferase family protein |
| DQ020644 | A_96_P054446 | 2,64 | up | rna polymerase ii largest subunit | F:transferase activity; C:nucleoplasm; F:DNA binding; P:transcription; | NP_195305 | AT4G35800 | NRPB1 | NRPB1_RNA_POL_II_LS_RNA_POL_II_LSRNA_POL_II_LS_RPB1__RNA polymerase II large |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | C:nucleolus; | | | | subunit |
| EF550528 | A_96_P013336 | 2,62 | up | s-adenosylmethionine decarboxylase proenzyme | P:cellular amino acid and derivative metabolic process; F:catalytic activity | NP_189184 | AT3G25570 | AT3G25570 | Adenosylmethionine decarboxylase family protein |
| BT013666 | A_96_P018211 | 2,62 | up | UVH3 (ULTRAVIOLET HYPERSENSITIVE 3 | F:nuclease activity; P:response to abiotic stimulus; P:response to stress; C:nucleus | NP_566830 | AT3G28030 | UVH3 | UVH3_UVR1__5'-3' exonuclease family protein |
| AK325264 | A_96_P077369 | 2,62 | up | cysteine protease | F:transcription factor activity; F:hydrolase activity; P:protein metabolic process; C:nucleoplasm | NP_568921 | AT5G60360 | AALP | AALP_ALP_SAG2__aleurain-like protease |
| TA37090_4081 | A_96_P226024 | 2,61 | up | protein | F:transferase activity; P:biosynthetic process; P:secondary metabolic process | NP_173653 | AT1G22360 | | UDP-glucosyl transferase 85A2 |
| BI422637 | A_96_P199644 | 2,60 | up | formate dehydrogenase | C:mitochondrion; P:response to abiotic stimulus; P:response to stress; C:membrane; C:endoplasmic reticulum; | NP_196982 | AT5G14780 | | Formate dehydrogenase |
| AK327264 | A_96_P071889 | 2,60 | up | pathogen induced protein 2-4 | F:protein binding; P:response to stress; P:response to abiotic stimulus | NP_850016 | AT2G21620 | RD2 | Adenine nucleotide alpha hydrolases-like superfamily protein |
| AK319264 | A_96_P084784 | 2,60 | up | transcription factor lhy | P:response to endogenous stimulus; P:response to abiotic stimulus; P:transcription; | NP_850460 | AT2G46830 | CCA1 | CCA1__circadian clock associated 1 |
| DQ100158 | A_96_P044446 | 2,58 | up | senescence-inducible chloroplast stay-green protein | P:cellular process; P:catabolic process | NP_567673 | AT4G22920 | | non-yellowing protein 1 |
| AK326455 | A_96_P240072 | 2,57 | up | wrky transcription factor 1 | F:DNA binding; P:transcription; F:transcription factor activity; C:nucleoplasm | NP_178199 | AT1G80840 | WRKY40 | ATWRKY40_WRKY40__WRKY DNA-binding protein 40 |
| DB684196 | A_96_P050686 | 2,52 | up | alpha-l-fucosidase 2 | C:cytoplasm | NP_174180 | AT1G28580 | AT1G28580 | GDSL-like Lipase/Acylhydrolase superfamily protein. |
| AK246404 | A_96_P020991 | 2,52 | up | polcalcin jun o 2 | C:mitochondrion; C:nucleus; P:response to stress; P:response to abiotic stimulus | NP_173866 | AT1G24620 | AT1G24620 | EF hand calcium-binding protein family |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| GO372514 | A_96_P074544 | 2,50 | up | fer_solly ame: full=ferredoxin | P:transport; C:plastid; P:generation of precursor metabolites and energy | NP_176291 | AT1G60950 | ATFD2 | ATFD2_FED A__2Fe-2S ferredoxin-like superfamily protein |
| AK247122 | A_96_P078114 | 2,50 | up | ethylene-regulated transcript 2 | C:nucleus | NP_193820 | AT4G20880 | | ethylene-responsive/regulated nuclear protein |
| DB698684 | A_96_P070694 | 2,50 | up | protein | F:transferase activity; P:cellular process; C:membrane | NP_194130 | AT4G23990 | | cellulose synthase-like protein G3 |
| BM410619 | A_96_P073764 | 2,49 | up | flavonol synthase flavanone 3- | P:metabolic process; P:biosynthetic process; P:secondary metabolic process | NP_181207 | AT2G36690 | AT2G36690 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein |
| DB686099 | A_96_P044581 | 2,48 | up | soul heme-binding family protein | C:cytoplasm; C:vacuole; F:binding; C:plasma membrane | NP_173153 | AT1G17100 | | |
| AK328540 | A_96_P090769 | 2,47 | up | flc-like 1 splice variant 4 | F:DNA binding; P:transcription | NP_201311 | AT5G65060 | FCL3 | K-box region and MADS-box transcription factor family protein |
| AK322251 | A_96_P229534 | 2,47 | up | transcription initiation factor iib | P:cellular component organization; P:transcription; C:ribosome; P:translation; C:nucleoplasm | NP_187644 | AT3G10330 | AT3G10330 | Cyclin-like family protein |
| DY523456 | A_96_P086424 | 2,46 | up | cytochrome p450 | C:cytoplasm; F:molecular_function; F:binding; P:metabolic process; F:oxygen binding; | NP_190421 | AT3G48520 | CYP94B3 | cytochrome P450, family 94, subfamily B, polypeptide 3 |
| AK324075 | A_96_P011461 | 2,44 | up | ac007591_30 ests gb | C:plastid; P:transport | NP_172989 | AT1G15370 | | SNARE-like protein |
| GO374313 | A_96_P036721 | 2,44 | up | cytochrome p450 | F:binding; P:metabolic process; F:catalytic activity; F:molecular_function | NP_196622 | AT5G10600 | CYP81K2 | CYP81K2__cytochrome P450 |
| X98308 | A_96_P012666 | 2,44 | up | myb-related transcription factor | P:transcription; C:nucleus; P:response to stress; P:response to abiotic stimulus | NP_188966 | AT3G23250 | MYB15 | ATMYB15_ATY19_MYB15__myb domain protein 15 |
| BG132464 | A_96_P189084 | 2,43 | up | atp synthase beta subunit | C:membrane; P:transport; F:hydrolase activity; F:transporter activity; C:thylakoid | NP_051066 | ATCG00480 | atpB | plant-specific TFIIB-related protein |
| BI209348 | A_96_P198349 | 2,43 | up | jasmonate ZIM-domain protein 3 | P:signal transduction; P:response to stress; P:response to biotic stimulus | NP_001078174 | AT3G17860 | JAZ3 | JAZIM3_ jasmonic acid mediated signaling pathway |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AK319494 | A_96_P194224 | 2,42 | up | thioredoxin m | F:enzyme regulator activity; P:cellular homeostasis; P:cell communication | NP_179159 | AT2G15570 | | thioredoxin M3 |
| AK324503 | A_96_P005516 | 2,41 | up | esterase lipase thioesterase family protein | F:hydrolase activity | NP_181474 | AT2G39420 | | alpha/beta-hydrolase domain-containing protein |
| BG124969 | A_96_P185104 | 2,41 | up | ac transposase | F:binding | NP_173291 | AT1G18560 | | BED zinc finger and hAT dimerization domain-containing protein |
| BI924398 | A_96_P201764 | 2,37 | up | zinc ion binding | C:intracellular; F:binding | NP_191626 | AT3G60670 | | PLATZ transcription factor family protein |
| AI489536 | A_96_P132342 | 2,35 | up | pectin methylesterase-like protein | P:cellular component organization; C:cell wall; C:plastid; C:plasma membrane; | NP_196538 | AT5G09760 | | putative pectinesterase/pectinesterase inhibitor 51 |
| EG553672 | A_96_P081084 | 2,34 | up | protease inhibitor family protein | C:cell; P:transport; F:lipid binding; F:hydrolase activity | NP_190966 | AT3G53980 | | bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin-like protein |
| AK321000 | A_96_P110407 | 2,34 | up | ap2 erf domain-containing transcription factor | F:binding; P:transport; P:cellular process; P:transcription | NP_194106 | AT4G23750 | | ethylene-responsive transcription factor CRF2 |
| AK320730 | A_96_P079544 | 2,33 | up | protein | F:hydrolase activity; P:carbohydrate metabolic process; C:cytoplasm; F:binding | NP_181976 | AT2G44480 | BGLU17 | beta glucosidase 17 |
| AK319191 | A_96_P249807 | 2,32 | up | Calmodulin binding | P:response to stress | NP_182147 | AT2G46240 | BAG6 | apoptosis response to H2O2to fungus stress response |
| AK326809 | A_96_P236723 | 2,31 | up | protein | F:molecular_function; P:biological_process; C:cellular_component | NP_564644 | AT1G53885 | | uncharacterized protein |
| AK326022 | A_96_P251942 | 2,31 | up | embryo-abundant protein | P:biological_process; P:metabolic process; C:mitochondrion; F:transferase activity | NP_181669 | AT2G41380 | AT2G41380 | S-adenosyl-L-methionine-dependent methyltransferases superfamily protein |
| AJ786362 | A_96_P021271 | 2,30 | up | protein | C:cytoplasm; P:lipid metabolic process; C:membrane; P:response to abiotic stimulus | NP_181340 | AT2G38050 | ATDET2 | ATDET2_DET2_DWF6__3-oxo-5-alpha-steroid 4-dehydrogenase family protein |
| BT014086 | A_96_P143966 | 2,30 | up | protein | F:binding | NP_565855 | AT2G36950 | | heavy-metal-associated domain-containing protein |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| GO374941 | A_96_P167039 | 2,29 | up | spermidine synthase | F:transferase activity; P:biosynthetic process; P:secondary metabolic process | NP_173794 | AT1G23820 | SPDS1 | SPDS1__spermidine synthase 1 |
| BG123989 | A_96_P106709 | 2,28 | up | abhydrolase domain | F:hydrolase activity | NP_564022 | AT1G17430 | | alpha/beta-hydrolase domain-containing protein |
| TA53412_4081 | A_96_P113112 | 2,28 | up | phytosulfokine peptide precursor | P:cell growth; F:receptor binding; C:extracellular region; P:signal transduction; | NP_566926 | AT3G49780 | | phytosulfokine-beta |
| AI488257 | A_96_P005126 | 2,28 | up | non-specific lipid transfer protein | P:response to stress; F:lipid binding; P:transport | NP_190727 | AT3G51590 | | non-specific lipid-transfer protein 12 |
| TA54771_4081 | A_96_P118882 | 2,28 | up | unconventional myosin | F:signal transducer activity; C:cytoskeleton; F:motor activity; C:membrane | NP_001154724 | AT5G20490 | | Myosin family protein with Dil domain |
| TA54720_4081 | A_96_P118667 | 2,27 | up | 60s ribosomal protein l37 | F:binding; F:structural molecule activity; C:ribosome; C:cytosol; P:translation; | NP_566535 | AT3G16080 | | 60S ribosomal protein L37-3 |
| BG127874 | A_96_P186484 | 2,25 | up | amp dependent | C:peroxisome; F:catalytic activity; F:nucleotide binding; | NP_564116 | AT1G20560 | AAE1 | acyl activating enzyme 1 |
| BG628739 | A_96_P192939 | 2,25 | up | unnamed protein product [Vitis vinifera] | | NP_199902 | AT5G50890 | AT5G50890 | alpha/beta-Hydrolases superfamily protein |
| AK323545 | A_96_P073749 | 2,25 | up | 4-coumarate: ligase | F:nucleotide binding; F:catalytic activity; P:secondary metabolic process | NP_188761 | AT3G21240 | 4CL2 | 4CL2_AT4CL2__4-coumarate:CoA ligase 2 |
| DV105470 | A_96_P247362 | 2,25 | up | mip tip subfamily | P:transport; P:cellular process; C:membrane; F:transporter activity | NP_181221 | AT2G36830 | GAMMA-TIP | GAMMA-TIP_GAMMA-TIP1_TIP1 |
| AK326213 | A_96_P080914 | 2,25 | up | wax synthase | P:biosynthetic process; P:cellular process; P:lipid metabolic process | NP_200151 | AT5G53390 | AT5G53390 | unknown |
| AK320112 | A_96_P184129 | 2,24 | up | hypothetical protein ARALYDRAFT | C:plastid | NP_193341 | AT4G16060 | | uncharacterized protein |
| AW738593 | A_96_P047556 | 2,24 | up | protein | C:cytoplasm; P:signal transduction; P:response to abiotic stimulus; P:transcription | NP_172923 | AT1G14700 | PAP3 | ATPAP3_PAP3__purple acid phosphatase 3 |
| AK324790 | A_96_P118107 | 2,23 | up | f-box protein | F:protein binding | NP_196378 | AT5G07610 | AT5G07610 | F-box and associated interaction domains-containing protein. |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AK224831 | A_96_P068621 | 2,23 | up | hydrogen peroxide-induced | P:biological_process; P:response to stress | NP_001078597 | AT5G17165 | | uncharacterized protein |
| DV105669 | A_96_P025741 | 2,22 | up | major latex-like protein | P:response to biotic stimulus; P:defense response; | NP_198153 | AT5G28010 | | SRPBCC ligand-binding domain-containing protein |
| AW455314 | A_96_P157461 | 2,21 | up | triacylglycerol lipase | F:hydrolase activity; C:mitochondrion; P:lipid metabolic process; | NP_193590 | AT4G18550 | | lipase class 3 family protein |
| TA55208_4081 | A_96_P120607 | 2,20 | up | wd40-repeat protein | C:intracellular; F:nucleotide binding | NP_188434 | AT3G18060 | | transducin/WD40 domain-containing protein |
| AK320937 | A_96_P097614 | 2,19 | up | acetyl- c-acetyltransferase | C:peroxisome; F:transferase activity; P:reproduction; P:lipid metabolic process | NP_568694 | AT5G48230 | ACAT2 | ACAT2_EMB1276__acetoacetyl-CoA thiolase 2 |
| AK323579 | A_96_P095189 | 2,18 | up | c- sterol isomerase | F:catalytic activity; C:membrane; C:endoplasmic reticulum; P:lipid metabolic process | NP_173433 | AT1G20050 | HYD1 | HYD1__C-8 cholestenol delta-isomerase |
| BE462099 | A_96_P060771 | 2,18 | up | tetracycline transporter | C:cytoplasm; F:transporter activity; C:membrane; P:transport | NP_179290 | AT2G16980 | AT2G16980 | Major facilitator superfamily protein |
| AY093595 | A_96_P012701 | 2,17 | up | osmotin-like protein | P:response to stress; P:response to biotic stimulus; C:vacuole; P:biological_process | NP_192902 | AT4G11650 | ATOSM34 | osmotin 34 |
| AY157061 | A_96_P232429 | 2,16 | up | protein | F:DNA binding; P:transcription; F:transcription factor activity; C:nucleoplasm | NP_174222 | AT1G29280 | WRKY65 | ATWRKY65_WRKY65__WRKY DNA-binding protein 65 |
| AK323147 | A_96_P230979 | 2,16 | up | cytochrome p450 | P:secondary metabolic process; F:catalytic activity; C:membrane; C:endoplasmic reticulum | NP_850337 | AT2G40890 | CYP98A3 | CYP98A3__cytochrome P450 |
| DV104023 | A_96_P246562 | 2,16 | up | cgi-144-like protein | C:cytoplasm | NP_564006 | AT1G16810 | | uncharacterized protein |
| TA43443_4081 | A_96_P180214 | 2,16 | up | pre-rrna-processing protein | C:nucleus; C:cytosol | NP_566132 | AT3G01160 | | uncharacterized protein |
| AK224691 | A_96_P072804 | 2,16 | up | jasmonate ZIM-domain protein 3 | P:signal transduction; P:response to stress; P:response to biotic stimulus | NP_566590 | AT3G17860 | JAZ3 | JAI3_JAZ3_TIFY6B__jasmonate-zim-domain protein 3 |
| BE431646 | A_96_P171999 | 2,16 | up | cinnamic acid 4-hydroxylase | F:binding; F:catalytic activity; P:secondary metabolic process | NP_180607 | AT2G30490 | ATC4H | ATC4H_C4H_CYP73A5_REF3__cinnamate-4-hydroxylase |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BI206635 | A_96_P025971 | 2,15 | up | inorganic pyrophosphatase | C:membrane; C:cytoplasm; C:nucleus; P:generation of precursor metabolites and energy | NP_171613 | AT1G01050 | AtPPa1 | pyrophosphorylase 1. |
| TA53991_4081 | A_96_P115502 | 2,15 | up | jasmonate ZIM-domain protein 1 | P:signal transduction; P:response to stress; P:response to biotic stimulus | NP_177395 | AT1G72510 | | uncharacterized protein |
| AK320874 | A_96_P056516 | 2,15 | up | cytochrome p450 | P:metabolic process; F:binding; F:catalytic activity | NP_195345 | AT4G36220 | CYP84A1 | CYP84A1_FAH1__ferulic acid 5-hydroxylase 1 |
| BW688800 | A_96_P067501 | 2,14 | up | PREDICTED: hypothetical protein [Vitis vinifera] | C:mitochondrion; F:molecular_function; P:biological_process | NP_563661 | AT1G02700 | | uncharacterized protein |
| BT012906 | A_96_P015157 | 2,14 | up | annexin 3 | P:response to stress; P:response to abiotic stimulus; C:cell; F:lipid binding; F:binding | NP_181410 | AT2G38760 | ANNAT3 | annexin 3 |
| AW443860 | A_96_P060181 | 2,14 | up | protein binding | C:cytoplasm; P:cell cycle; F:nucleotide binding; P:chromosome segregation; P:cell division; | NP_191909 | AT3G63500 | | uncharacterized protein |
| BI207682 | A_96_P197724 | 2,14 | up | late blight resistance identical | P:cell death; P:response to stress; F:nucleotide binding | NP_001077768 | AT1G63750 | | TIR-NBS-LRR class disease resistance protein |
| AK323093 | A_96_P020541 | 2,14 | up | hydrophobic low temperature and salt responsive | P:response to stress; P:response to abiotic stimulus; C:cytoplasm; P:response to biotic stimulus; C:membrane | NP_194795 | AT4G30660 | AT4G30660 | Low temperature and salt responsive protein family |
| AK322781 | A_96_P181229 | 2,12 | up | auxin-responsive protein | P:transcription; P:response to endogenous stimulus; P:signal transduction; C:nucleus | NP_193191 | AT4G14550 | IAA14 | IAA14_SLR__indole-3-acetic acid inducible 14 |
| AK328581 | A_96_P121587 | 2,12 | up | auxin-induced protein | P:secondary metabolic process; C:cytoplasm; P:response to stress; P:response to biotic stimulus; P:signal transduction; | NP_187538 | AT3G09270 | ATGSTU8 | glutathione S-transferase TAU 8 |
| AI485590 | A_96_P130647 | 2,12 | up | cucumber peeling | C:cytoplasmic membrane-bounded vesicle; F:electron carrier activity; F:copper ion binding | NP_566810 | AT3G27200 | AT3G27200 | Cupredoxin superfamily protein. |
| AK325600 | A_96_P060576 | 2,12 | up | cytochrome p450 | F:binding; P:metabolic process; F:molecular_function; F:catalytic activity | NP_182081 | AT2G45570 | CYP76C2 | cytochrome P450, family 76, subfamily C, polypeptide 2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ES893872 | A_96_P074162 | 2,11 | up | glutathione s-transferase | P:signal transduction; P:response to stress; P:carbohydrate metabolic process; | NP_565178 | AT1G78380 | GSTU19 | ATGSTU19_GST8_GSTU19__glutathione S-transferase TAU 19 |
| BP886864 | A_96_P213349 | 2,11 | up | atp-binding cassette superfamily | F:hydrolase activity; F:transporter activity; C:plasma membrane; F:nucleotide binding; | NP_850354 | AT2G41700 | ABCA1 | ABCA1_AtABCA1__ATP-binding cassette A1 |
| AK320297 | A_96_P131162 | 2,10 | up | phosphatidylcholine-sterol o-acyltransferase | C:cytoplasm; F:transferase activity; P:lipid metabolic process; P:biosynthetic process | NP_171897 | AT1G04010 | | phospholipid sterol acyl transferase 1 |
| DB684300 | A_96_P059491 | 2,09 | up | leucine rich | C:cytoplasm; F:kinase activity | NP_197548 | AT5G20480 | EFR | EFR__EF-TU receptor |
| AI774144 | A_96_P134882 | 2,09 | up | calcium binding protein | F:binding | NP_187405 | AT3G07490 | AGD11 | ARF-GAP domain 11. |
| AK329228 | A_96_P116807 | 2,08 | up | zinc ion binding protein | F:zinc ion binding; P:biological_process; F:protein binding; C:cellular_component | NP_563644 | AT1G02070 | | uncharacterized protein |
| DV104534 | A_96_P246722 | 2,08 | up | protein | C:cytoplasm; C:membrane; C:cell wall; C:Golgi apparatus; P:transport; C:plasma membrane | NP_198547 | AT5G37310 | AT5G37310 | Endomembrane protein 70 protein family |
| AK319313 | A_96_P245365 | 2,07 | up | protein | P:protein modification process; F:nucleotide binding; C:cytosol; F:kinase activity; | NP_176925 | AT1G67580 | AT1G67580 | Protein kinase superfamily protein |
| BI934065 | A_96_P065106 | 2,07 | up | late embryogenesis abundant domain-containing protein | P:response to stress; P:response to biotic stimulus; P:response to abiotic stimulus; | NP_567398 | AT4G13230 | | late embryogenesis abundant domain-containing protein |
| AI898304 | A_96_P140962 | 2,06 | up | nucleic acid binding | F:DNA binding; P:transcription | NP_850678 | AT3G51620 | | NT domain of poly(A) polymerase and terminal uridylyl transferase-containing protein |
| BG631546 | A_96_P004906 | 2,06 | up | family ii extracellular lipase 1 | F:transferase activity; C:extracellular region; F:hydrolase activity; P:lipid metabolic process | NP_565120 | AT1G75880 | AT1G75880 | SGNH hydrolase-type esterase superfamily protein |
| BP897734 | A_96_P217894 | 2,05 | up | 14-3-3 protein | F:protein binding | NP_564249 | AT1G26480 | GRF12 | GF14 IOTA_GRF12__general regulatory factor 12 |
| TA53364_4081 | A_96_P112897 | 2,05 | up | udp-glucose:flavonoid glucoside - glucosyltransferase | P:metabolic process; F:transferase activity | NP_192016 | AT4G01070 | | hydroquinone glucosyltransferase |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AW625828 | A_96_P034406 | 2,04 | up | saur33 - auxin-responsive saur family member | P:response to endogenous stimulus; P:signal transduction; C:plastid; C:mitochondrion; | NP_197582 | AT5G20820 | | SAUR-like auxin-responsive protein family |
| AK325632 | A_96_P128122 | 2,04 | up | lipase class 3 family protein | F:hydrolase activity; C:membrane; P:catabolic process; P:lipid metabolic process; | NP_563660 | AT1G02660 | | alpha/beta-hydrolase domain-containing protein |
| BG791295 | A_96_P196029 | 2,04 | up | protein | F:transferase activity; P:metabolic process | NP_173652 | AT1G22340 | AtUGT85A7 | UDP-glucosyl transferase 85A7 |
| AK325559 | A_96_P043126 | 2,04 | up | f-box kelch repeat-containing f-box family protein | C:membrane | NP_564193 | AT1G23390 | | Kelch repeat-containing F-box protein |
| DB712076 | A_96_P069879 | 2,04 | up | vacuolar sorting | P:transport; P:cellular process | NP_179370 | AT2G17790 | VPS35A | VPS35A_ZIP3__VPS35 homolog |
| BI205334 | A_96_P086144 | 2,03 | up | gh3 family protein | F:catalytic activity; P:biological_process; P:response to endogenous stimulus | NP_179898 | AT2G23170 | GH3.3 | Auxin-responsive GH3 family protein |
| AK322664 | A_96_P060356 | 2,03 | up | hd domain class transcription factor | P:signal transduction; P:response to stress; P:response to abiotic stimulus; P:transcription; C:nucleoplasm | NP_565536 | AT2G22430 | ATHB6 | ATHB6_HB6__homeobox protein 6 |
| BG131595 | A_96_P119432 | 2,03 | up | pentatricopeptide repeat-containing | C:plastid; C:mitochondrion | NP_193307 | AT4G15720 | AT4G15720 | Tetratricopeptide repeat (TPR)-like superfamily protein. |
| BI926661 | A_96_P203209 | 2,02 | up | atp binding | C:cytoplasm | NP_001117517 | AT1G59540 | ZCF125 | ZCF125__P-loop containing nucleoside triphosphate hydrolases superfamily protein |
| TA41248_4081 | A_96_P079089 | 2,02 | up | protein phosphatase 2c | P:signal transduction; P:response to stress; P:response to abiotic stimulus; F:hydrolase activity; | NP_172223 | AT1G07430 | | protein phosphatase 2C 3 |
| AK330010 | A_96_P046946 | 2,02 | up | esterase lipase thioesterase family protein | F:catalytic activity | NP_175685 | AT1G52760 | | lysophospholipase 2 |
| AK319742 | A_96_P052056 | 2,01 | up | cxe carboxylesterase | P:metabolic process; F:hydrolase activity | NP_190439 | AT3G48700 | | carboxyesterase 13 |
| FS195438 | A_96_P125062 | 2,01 | up | protein | F:RNA binding; C:mitochondrion; F:nucleotide binding | NP_568464 | AT5G25060 | AT5G25060 | RNA recognition motif (RRM)-containing protein |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AK321499 | A_96_P078104 | 2,01 | up | protein | F:nucleotide binding; F:kinase activity; P:cellular amino acid and derivative metabolic process | NP_178651 | AT2G05940 | AT2G05940 | Protein kinase superfamily protein |
| AK319311 | A_96_P080504 | 2,01 | up | casein kinase | F:nucleotide binding; F:kinase activity; C:plastid; C:mitochondrion; P:cellular amino acid and derivative metabolic process | NP_180147 | AT2G25760 | | casein kinase I-like protein |
| BM411460 | A_96_P240283 | 2,01 | up | phenylcoumaran benzylic ether reductase 3 | P:metabolic process; F:catalytic activity; C:cytoplasm; F:binding | NP_195634 | AT4G39230 | AT4G39230 | NmrA-like negative transcriptional regulator family protein |
| BT013144 | A_96_P182679 | 2,01 | up | rubisco subunit binding-protein alpha subunit | C:mitochondrion; C:plastid; P:cellular component organization; F:nucleotide binding; F:protein binding; C:ribosome; C:membrane; C:extracellular region | NP_180367 | AT2G28000 | CH-CPN60A | CH-CPN60A_CPN60A_SLP__chaperonin-60alpha |
| AK324517 | A_96_P232794 | 2,01 | up | anthocyanin 5-o-glucosyltransferase | F:transferase activity; P:metabolic process | NP_567471 | AT4G15550 | IAGLU | indole-3-acetate beta-D-glucosyltransferase |
| AF179247 | A_96_P011901 | 9,32 | down | 1-aminocyclopropane-1-carboxylate synthase | P:biosynthetic process; F:binding; P:ripening; F:transferase activity; F:catalytic activity | NP_195491 | AT4G37770 | ACS8 | ACS8__1-amino-cyclopropane-1-carboxylate synthase 8 |
| BF050158 | A_96_P179674 | 6,95 | down | rna-dependent rna polymerase family protein | P: transcription; F: DNA binding | NP_179583 | AT2G19930 | | RNA-dependent RNA polymerase-like protein |
| TA36926_4081 | A_96_P090309 | 6,43 | down | protein | C:nucleus; F:hydrolase activity; P:protein metabolic process; C:membrane; C:plastid; C:thylakoid | NP_194120 | AT4G23890 | | uncharacterized protein |
| AK247747 | A_96_P055431 | 4,79 | down | bzip transcription factor | P:transcription; F:DNA binding; C:cytoplasm; F:protein binding; C:nucleoplasm | NP_177031 | AT1G68640 | PAN | PAN__bZIP transcription factor family protein |
| TA53803_4081 | A_96_P205009 | 4,56 | down | eix receptor 2 | F:receptor activity; F:protein binding; P:signal transduction | NP_181039 | AT2G34930 | | disease resistance-like protein/LRR domain-containing protein |
| BP894496 | A_96_P216484 | 4,21 | down | aspartyl protease family protein | C:cell; P:protein metabolic process; P:catabolic process; F:hydrolase activity | NP_188478 | AT3G18490 | AT3G18490 | Eukaryotic aspartyl protease family protein |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AW649231 | A_96_P161871 | 4,12 | down | 2-oxoglutarate-dependent dioxygenase | P:carbohydrate metabolic process; P:secondary metabolic process; F:catalytic activity | NP_175689 | AT1G52800 | AT1G52800 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein |
| X71592 | A_96_P077804 | 3,97 | down | pathogenesis-related protein 1 | C:extracellular region; P:response to stress; P:response to biotic stimulus | NP_188603 | AT3G19690 | AT3G19690 | PR1 |
| AK328875 | A_96_P204784 | 3,81 | down | xenotropic and polytropic murine leukemia virus receptor ids- | P:response to extracellular stimulus; P:cell communication; P:response to stress | NP_568312 | AT5G15330 | ATSPX4 | SPX domain gene 4 |
| CK714884 | A_96_P226109 | 3,79 | down | at1g68530 t26j14_10 | P:response to abiotic stimulus; P:response to stress; P:anatomical structure morphogenesis; C:membrane; C:endoplasmic reticulum; C:cytosol | NP_173916 | AT1G25450 | | 3-ketoacyl-CoA synthase 5 |
| BT014084 | A_96_P003801 | 3,69 | down | protein | C:plastid | NP_194974 | AT4G32480 | | uncharacterized protein |
| TA47801_4081 | A_96_P252767 | 3,58 | down | | F:molecular_function; P:biological_process; C:cellular_component | NP_565715 | AT2G31130 | | uncharacterized protein |
| DY523815 | A_96_P248207 | 3,42 | down | 1-aminocyclopropane-1-carboxylate oxidase | P:metabolic process; F:catalytic activity | NP_192787 | AT4G10490 | AT4G10490 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein |
| BF097766 | A_96_P086219 | 3,24 | down | cytochrome p450 | F:binding; P:metabolic process; F:catalytic activity; F:molecular_function | NP_179995 | AT2G24180 | CYP71B6 | CYP71B6__cytochrome p450 71b6 |
| AK327314 | A_96_P264462 | 3,21 | down | pfkb-type carbohydrate kinase family protein | F:kinase activity; C:plastid; P:catabolic process; P:secondary metabolic process | NP_199996 | AT5G51830 | AT5G51830 | pfkB-like carbohydrate kinase family protein |
| AK320058 | A_96_P227299 | 3,17 | down | protein | P:lipid metabolic process; F:triglyceride lipase activity | NP_199107 | AT5G42930 | | lipase class 3-like protein |
| AK321656 | A_96_P170809 | 3,08 | down | protein | C:plasma membrane | NP_191207 | AT3G56480 | | myosin heavy chain-like protein |
| DV103959 | A_96_P246512 | 3,07 | down | heat shock protein | F:DNA binding | NP_188922 | AT3G22830 | AT-HSFA6B | AT-HSFA6B_HSFA6B__heat shock transcription factor A6B |
| BP908408 | A_96_P038696 | 3,06 | down | early-responsive to dehydration expressed | C:membrane; C:cytoplasm | NP_172480 | AT1G10090 | AT1G10090 | Early-responsive to dehydration stress protein (ERD4) |
| AK326433 | A_96_P178449 | 3,04 | down | pectate lyase | F:catalytic activity; | NP_567707 | AT4G24780 | | putative pectate lyase 18 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | C:membrane | | | | |
| BI924133 | A_96_P201549 | 3,02 | down | set domain protein | P:transcription; P:cellular component organization; P:anatomical structure morphogenesis; C:cytoplasm; C:nucleus; C:plasma membrane | NP_172074 | AT1G05830 | ATX2 | ATX2_SDG30__trithorax-like protein 2 |
| AK322569 | A_96_P061441 | 2,85 | down | cytochrome p450 | F:binding; F:catalytic activity | NP_194922 | AT4G31940 | CYP82C4 | cytochrome P450, family 82, subfamily C, polypeptide 4 |
| AK320454 | A_96_P048876 | 2,84 | down | protein | C:cell wall | NP_196703 | AT5G11420 | AT5G11420 | unknown |
| DB710487 | A_96_P036911 | 2,83 | down | beta-galactosidase like protein | F:binding; P:carbohydrate metabolic process; P:cellular process; P:lipid metabolic process; | NP_849506 | AT4G36360 | BGAL3 | beta-galactosidase 3. |
| AK327173 | A_96_P238757 | 2,80 | down | protein | P:response to endogenous stimulus; P:biological_process; F:binding | NP_189461 | AT3G28210 | | zinc finger AN1 domain-containing stress-associated protein 12 |
| AW626100 | A_96_P160701 | 2,77 | down | myb transcription factor | P:transcription; P:cellular process; P:response to endogenous stimulus; P:reproduction; C:nucleoplasm | NP_172425 | AT1G09540 | MYB61 | ATMYB61_MYB61__myb domain protein 61 |
| AK327172 | A_96_P121022 | 2,75 | down | protein | F:molecular_function; P:biological_process; C:cellular_component | NP_172667 | AT1G12030 | | uncharacterized protein |
| BP908452 | A_96_P221699 | 2,68 | down | cationic amino acid transporter | P:response to biotic stimulus; F:transporter activity; C:plasma membrane; C:membrane | NP_187671 | AT3G10600 | CAT7 | cationic amino acid transporter 7 |
| AW979912 | A_96_P170324 | 2,65 | down | cytochrome p450 | C:cytoplasm; F:binding; F:oxygen binding; C:endoplasmic reticulum; C:plastid | NP_182075 | AT2G45510 | CYP704A2 | cytochrome P450, family 704, subfamily A, polypeptide 2 |
| AK329117 | A_96_P246627 | 2,64 | down | phloem protein | C:cell; F:carbohydrate binding | NP_683296 | AT1G10155 | | phloem protein 2-A10 |
| BP893421 | A_96_P216104 | 2,64 | down | protein | P:transport; P:cellular process; C:membrane; F:transporter activity | NP_177332 | AT1G71870 | AT1G71870 | MATE efflux family protein |
| AW035415 | A_96_P038996 | 2,63 | down | branched-chain amino acid aminotransferase | F:transferase activity; C:plastid; C:mitochondrion; P:catabolic process; | NP_172478 | AT1G10070 | ATBCAT-2 | ATBCAT-2_BCAT-2__branched-chain amino acid transaminase 2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AK321314 | A_96_P048831 | 2,61 | down | disease resistance-responsive family protein fibroin-related | F:binding; C:cytoplasm | NP_973782 | AT1G07730 | | disease resistance-responsive, dirigent domain-containing protein |
| AK319223 | A_96_P070704 | 2,60 | down | mudrA protein-maize transposon MuDR | F:nucleic acid binding | NP_175414 | AT1G49920 | | MuDR family transposase |
| AI782347 | A_96_P138892 | 2,60 | down | zinc finger (b-box type) family protein | P:transcription; C:plasma membrane; F:transcription factor activity; F:binding; C:nucleoplasm | NP_176986 | AT1G68190 | | putative zinc finger protein |
| AK324806 | A_96_P233139 | 2,56 | down | nodulin 21 family protein | C:cell | NP_177183 | AT1G70260 | | nodulin MtN21 /EamA-like transporter protein |
| AW623437 | A_96_P159071 | 2,54 | down | inosine-uridine preferring nucleoside | C:cytoplasm; F:hydrolase activity; C:cell wall; C:mitochondrion | NP_197387 | AT5G18860 | | inosine-uridine preferring nucleoside hydrolase family protein |
| AK323186 | A_96_P230709 | 2,51 | down | protein | C:plastid; F:molecular_function; P:biological_process | NP_565220 | AT1G79770 | | uncharacterized protein |
| X98929 | A_96_P000061 | 2,49 | down | subtilisin-like protease | F:protein binding; C:cell wall; F:hydrolase activity; C:cytoplasm; C:extracellular region | NP_569048 | AT5G67360 | | subtilisin-like protease |
| TA56692_4081 | A_96_P126712 | 2,47 | down | heat shock protein binding | P:protein metabolic process; P:cellular process | NP_564717 | AT1G56300 | | chaperone DnaJ-domain containing protein |
| AK325623 | A_96_P234029 | 2,46 | down | protein | C:cell; C:cytoplasm; C:vacuole; C:cell wall | NP_196799 | AT5G12950 | | uncharacterized protein |
| AW036000 | A_96_P030741 | 2,45 | down | sulfate bicarbonate oxalate exchanger and transporter sat-1 | C:membrane; F:transporter activity; P:transport; P:cellular process | NP_190758 | AT3G51895 | SULTR3 | sulfate transporter |
| AK320138 | A_96_P031762 | 2,43 | down | glycosyl group 1 family protein | P:response to extracellular stimulus; P:cell communication; P:response to stress; C:plastid | NP_568085 | AT5G01220 | | sulfoquinovosyldiacylglycerol 2 |
| AF179249 | A_96_P095644 | 2,42 | down | 1-aminocyclopropane-1-carboxylate synthase | F:transferase activity; F:binding; P:biosynthetic process; F:catalytic activity; | NP_192867 | AT4G11280 | ACS6 | ACS6_ATACS6__1-aminocyclopropane-1-carboxylic acid (acc) synthase 6 |
| BF097299 | A_96_P181749 | 2,40 | down | protein | C:cytoplasm; F:hydrolase activity; P:protein metabolic process; C:extracellular region | NP_564107 | AT1G20160 | | Subtilisin-like serine endopeptidase-like protein |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| DB718737 | A_96_P209849 | 2,40 | down | protein | F:catalytic activity | NP_197540 | AT5G20400 | AT5G20400 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein |
| BG130811 | A_96_P026736 | 2,38 | down | phosphoenolpyruvate carboxylase | C:cytoplasm; P:catabolic process; P:photosynthesis; P:carbohydrate metabolic process; | NP_175738 | AT1G53310 | ATPEPC1 | phosphoenolpyruvate carboxylase 1 |
| DB716608 | A_96_P067916 | 2,38 | down | sterol regulatory element-binding protein site 2 | P:response to abiotic stimulus; P:response to stress; C:membrane; C:plastid | NP_173229 | AT1G17870 | | ethylene-dependent gravitropism-deficient and yellow-green-like 3 protein |
| AK319877 | A_96_P220429 | 2,37 | down | ferric reductase-like transmembrane component | C:plasma membrane; F:binding; P:photosynthesis; C:membrane; C:plastid; P:response to abiotic stimulus | NP_199784 | AT5G49730 | | ferric reduction oxidase 6 |
| BG130117 | A_96_P064386 | 2,36 | down | swim zinc finger family protein | F:binding | NP_974054 | AT1G60560 | | SWIM zinc finger-like protein |
| AK328502 | A_96_P229454 | 2,36 | down | bim1 dna binding protein binding transcription factor | P:response to endogenous stimulus; P:signal transduction; C:nucleoplasm; P:transcription | NP_001119190 | AT5G08130 | BIM1 | BIM1__basic helix-loop-helix (bHLH) DNA-binding superfamily protein |
| AI777995 | A_96_P033561 | 2,36 | down | protein | P:biosynthetic process; P:cellular process; P:lipid metabolic process | NP_194433 | AT4G27030 | | fatty acid desaturase A |
| DB683870 | A_96_P174494 | 2,34 | down | serine acetyltransferase | C:cytosol; F:binding; C:mitochondrion; P:response to stress; P:response to abiotic stimulus; P:cell communication; C:plastid | NP_187918 | AT3G13110 | SAT-A | serine acetyltransferase 3 |
| AK324190 | A_96_P232399 | 2,34 | down | glutamate-gated kainate-type ion channel receptor subunit 5 | P:transport; F:receptor activity; C:membrane; C:external encapsulating structure; P:cell-cell signaling; P:signal transduction | NP_180475 | AT2G29110 | ATGLR2.8 | ATGLR2.8_GLR2.8_GLR2.8__glutamate receptor 2.8 |
| BG127331 | A_96_P033651 | 2,33 | down | gh3-like protein | P:response to endogenous stimulus; P:response to abiotic stimulus | NP_192249 | AT4G03400 | | auxin-responsive GH3 family protein |
| U13055 | A_96_P245140 | 2,33 | down | endo- -beta-glucanase precursor | F:hydrolase activity; P:cellular component organization; P:cell growth; P:abscission; | NP_177228 | AT1G70710 | CEL1 | glycosyl hydrolase 9B1 |
| AK325209 | A_96_P064151 | 2,33 | down | constans 1 | F:binding; C:intracellular | NP_197089 | AT5G15850 | ATCOL1 | ATCOL1_COL1__CONSTANS-like 1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AK324830 | A_96_P246702 | 2,32 | down | beta-glucosidase 47 | P:carbohydrate metabolic process; F:hydrolase activity; F:binding; C:cytoplasm; P:secondary metabolic process | NP_193907 | AT4G21760 | BGLU47 | beta-glucosidase 47 |
| AW738136 | A_96_P164401 | 2,31 | down | adaptin family protein | C:Golgi apparatus; F:protein binding; C:membrane; C:cytoplasm; P:transport; P:cellular component organization | NP_974443 | AT3G55480 | PAT2 | PAT2__protein affected traf&#64257 |
| BM410928 | A_96_P043161 | 2,31 | down | nac domain ipr003441 | P:response to stress; P:transcription; F:protein binding; C:nucleoplasm | NP_188170 | AT3G15510 | | NAC domain containing protein 2 |
| AK320732 | A_96_P184239 | 2,28 | down | chitinase 134 | F:hydrolase activity; C:extracellular space; P:carbohydrate metabolic process; P:response to stress; | NP_566426 | AT3G12500 | | chitinase |
| AK323358 | A_96_P003831 | 2,28 | down | protein | C:plasma membrane | NP_177935 | AT1G78110 | | uncharacterized protein |
| AK327977 | A_96_P218029 | 2,25 | down | cbs domain-containing protein | P:response to stress; P:response to abiotic stimulus; C:mitochondrion; F:binding | NP_196647 | AT5G10860 | AT5G10860 | Cystathionine beta-synthase (CBS) family protein |
| AK320322 | A_96_P227554 | 2,25 | down | malate synthase | F:transferase activity; P:generation of precursor metabolites and energy; P:catabolic process; C:peroxisome; | NP_196006 | AT5G03860 | MLS | MLS__malate synthase |
| AK324526 | A_96_P042041 | 2,22 | down | protein | P:carbohydrate metabolic process; C:extracellular region; F:hydrolase activity; | NP_199747 | AT5G49360 | BXL1 | beta-xylosidase 1 |
| AK325889 | A_96_P171069 | 2,22 | down | protein | F:binding; P:response to extracellular stimulus; P:cell communication; P:response to stress | NP_199696 | AT5G48850 | | tetratricopeptide repeat domain-containing protein |
| BG134199 | A_96_P190219 | 2,21 | down | major intrinsic protein | P:transport; P:cellular process; C:membrane; F:transporter activity | NP_193626 | AT4G18910 | ATNLM2 | ATNLM2_NIP1 |
| AF154003 | A_96_P012826 | 2,20 | down | pirin-like protein | C:nucleus; C:mitochondrion; C:plastid | NP_850385 | AT2G43120 | AT2G43120 | Pirin-like protein |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BG135790 | A_96_P191179 | 2,19 | down | dicer-like protein | F:nuclease activity; F:RNA binding; P:regulation of gene expression, epigenetic; P:response to stress; P:response to biotic stimulus | NP_566199 | AT3G03300 | ATDCL2 | ATDCL2_DCL2__dicer-like 2 |
| AK320574 | A_96_P058886 | 2,19 | down | high affinity nitrate transporter | C:membrane; C:vacuole; P:transport; P:cellular process; F:transporter activity | NP_196961 | AT5G14570 | ATNRT2.7 | ATNRT2.7_NRT2.7__high affinity nitrate transporter 2.7 |
| AK247493 | A_96_P171789 | 2,18 | down | leucine-rich repeat family protein | C:plasma membrane; P:response to biotic stimulus; F:protein binding; P:signal transduction; C:plastid | NP_564942 | AT1G68780 | AT1G68780 | RNI-like superfamily protein |
| BT013433 | A_96_P259447 | 2,17 | down | inducer of cbf expression 1 | F:DNA binding; P:response to stress; P:response to abiotic stimulus; C:nucleus; P:transcription | NP_189309 | AT3G26744 | ATICE1 | ATICE1_ICE1_SCRM__basic helix-loop-helix (bHLH) DNA-binding superfamily protein |
| BI927023 | A_96_P027451 | 2,17 | down | chalcone synthase family protein | P:anatomical structure morphogenesis; P:cellular component organization; P:secondary metabolic process | NP_567971 | AT4G34850 | | Chalcone and stilbene synthase family protein |
| AK323674 | A_96_P231489 | 2,16 | down | delta-12 oleate desaturase | P:metabolic process; F:catalytic activity; P:lipid metabolic process | NP_187819 | AT3G12120 | | omega-6 fatty acid desaturase, endoplasmic reticulum |
| BT014116 | A_96_P222569 | 2,16 | down | acr3 amino acid binding | P:metabolic process; C:cytosol; F:binding | NP_565146 | AT1G76990 | | ACT domain-containing protein 3 |
| AK324231 | A_96_P052956 | 2,15 | down | lipid binding | C:cytoplasm; C:mitochondrion | NP_568699 | AT5G48485 | | bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin-like protein |
| AB004558 | A_96_P011826 | 2,15 | down | cell-wall invertase | C:extracellular region; F:hydrolase activity; C:cell wall; P:carbohydrate metabolic process; | NP_190828 | AT3G52600 | CWIN2 | cell wall invertase 2 |
| AK321479 | A_96_P163046 | 2,14 | down | cytochrome p450 | F:binding; P:metabolic process; F:catalytic activity; F:molecular_function | NP_198460 | AT5G36110 | CYP716A1 | cytochrome P450, family 716, subfamily A, polypeptide 1. |
| AK322310 | A_96_P103969 | 2,12 | down | glucosyl transferase | F:transferase activity; P:metabolic process | NP_172044 | AT1G05530 | AT1G05530 | UDP-glucosyl transferase 75B2 |
| AK324313 | A_96_P056716 | 2,12 | down | beta-cyanoalanine synthase | P:response to stress; C:mitochondrion; P:cellular process; F:transferase activity; C:cytoplasm | NP_191703 | AT3G61440 | CYSC1 | cysteine synthase C1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AW615915 | A_96_P157571 | 2,12 | down | potyviral helper component protease-interacting protein 1 | F:binding; F:protein binding; F:hydrolase activity | NP_192036 | AT4G01270 | AT4G01270 | RING/U-box superfamily protein |
| BT012876 | A_96_P254477 | 2,11 | down | protein | C:membrane; C:Golgi apparatus; F:protein binding; P:transport; P:cellular process; F:binding; | NP_565651 | AT2G27460 | | sec23/sec24 transport family protein |
| TA36796_4081 | A_96_P089969 | 2,11 | down | protein | P:cellular component organization;P:response to biotic stimulus; P:response to abiotic stimulus; | NP_567759 | AT4G26850 | | GDP-L-galactose phosphorylase |
| AK328261 | A_96_P068741 | 2,11 | down | gibberellin 2-oxidase | P:metabolic process; F:binding;P:lipid metabolic process; P:secondary metabolic process | NP_001031112 | AT1G30040 | GA2OX2 | gibberellin 2-oxidase |
| AF096776 | A_96_P012926 | 2,11 | down | expansin | C:extracellular region; P:cellular component organization; P:cellular process; C:membrane | NP_181593 | AT2G40610 | | expansin A8 |
| BF098346 | A_96_P182694 | 2,09 | down | pho1-like protein | P:transport; P:response to extracellular stimulus; P:cell communication; P:response to stress | NP_188985 | AT3G23430 | | phosphate transporter PHO1 |
| DB724515 | A_96_P059476 | 2,08 | down | nucleotide binding protein | C:cytosol; F:binding; F:nucleotide binding; F:protein binding | NP_195154 | AT4G34280 | | transducin/WD40 domain-containing protein |
| TA47182_4081 | A_96_P260397 | 2,08 | down | cyclic nucleotide-gated ion | P:transport; P:cellular process; F:transporter activity; F:protein binding; C:membrane | NP_200125 | AT5G53130 | | cyclic nucleotide-gated ion channel 1 |
| AW219022 | A_96_P046311 | 2,07 | down | mads-box protein | P:transport; P:transcription; P:response to stress; P:response to abiotic stimulus; F:DNA binding; F:transcription regulator activity; F:protein binding; F:transcription factor activity; C:cytoplasm; C:nucleoplasm | NP_182090 | AT2G45660 | AGL20 | AGL20_ATSOC1_SOC1__AGAMOUS-like 20 |
| TA37481_4081 | A_96_P172969 | 2,07 | down | 4-coumarate- ligase-like protein | F:nucleotide binding; C:plastid; C:extracellular region; F:catalytic activity; | NP_190468 | AT3G48990 | | AMP-dependent synthetase and ligase-like protein |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BP897166 | A_96_P259463 | 2,07 | down | ribulose bisphosphate carboxylase | P:biosynthetic process; P:photosynthesis;P:cellular process; F:protein binding; C:plastid | NP_198657 | AT5G38410 | AT5G38410 | Ribulose bisphosphate carboxylase (small chain) family protein |
| TA56280_4081 | A_96_P125022 | 2,05 | down | hexose transporter | P:transport; P:cellular process; F:transporter activity; C:membrane; F:catalytic activity | NP_197997 | AT5G26250 | | sugar transport protein 8 |
| BG133784 | A_96_P189949 | 2,05 | down | protein | F:kinase activity | NP_192232 | AT4G03230 | AT4G03230 | G-type lectin S-receptor-like serine/threonine-protein kinase |
| TA36295_4081 | A_96_P088464 | 2,04 | down | glyceraldehyde-3-phosphate dehydrogenase | F:nucleotide binding;P:carbohydrate metabolic process; P:generation of precursor metabolites and energy; | NP_001117276 | AT1G12900 | | glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) |
| AK327122 | A_96_P017621 | 2,04 | down | regulator of chromosome condensation family protein | F:kinase activity; F:chromatin binding; F:protein binding; C:intracellular | NP_191117 | AT3G55580 | AT3G55580 | Regulator of chromosome condensation (RCC1) family protein |
| BE450553 | A_96_P044361 | 2,03 | down | dna binding | F:DNA binding | NP_564236 | AT1G25550 | AT1G25550 | MYB TF |
| BM411846 | A_96_P042286 | 2,03 | down | microtubule-associated protein | P:response to stress; P:response to abiotic stimulus; P:cellular component organization; P:signal transduction; C:cytoskeleton | NP_198861 | AT5G40450 | AT5G40450 | unknown |
| BG123415 | A_96_P184454 | 2,03 | down | fructose- -bisphosphatase | C:cytoplasm; P:carbohydrate metabolic process; P:generation of precursor metabolites and energy; P:secondary metabolic process | NP_175032 | AT1G43670 | AT1G43670 | Inositol monophosphatase family protein |
| AK327992 | A_96_P230894 | 2,02 | down | mads box | P:multicellular organismal development; F:DNA binding; P:transcription; C:nucleoplasm | NP_001077873 | AT2G03060 | | protein agamous-like 30 |
| AK328892 | A_96_P055806 | 2,02 | down | protein | C:cytoplasm; F:receptor activity; C:nucleus; P:signal transduction; F:lipid binding | NP_565887 | AT2G38310 | AT2G38310 | ABA signaling abiotic and biotic stress response |
| AK326775 | A_96_P092354 | 2,02 | down | aldehyde dehydrogenase | F:nucleotide binding; C:mitochondrion; P:generation of precursor metabolites and energy; P:secondary metabolic process | NP_190383 | AT3G48000 | ALDH2 | ALDH2_ALDH2A_ALDH2B4__aldehyde dehydrogenase 2B4 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AW625874 | A_96_P160571 | 2,02 | down | arabinogalactan protein | C:cytosol; C:mitochondrion; C:plasma membrane | NP_196729 | AT5G11680 | AT5G11680 | FUNCTIONS IN: molecular_function unknown |
| DB681453 | A_96_P058301 | 2,02 | down | nodulin family protein | C:cell | NP_177616 | AT1G74780 | | nodulin-like and major facilitator domain-containing protein |
| TA56993_4081 | A_96_P127927 | 2,02 | down | protein | F:binding | NP_973537 | AT2G26695 | | Ran BP2/NZF zinc finger-like protein |
| AK325814 | A_96_P063191 | 2,01 | down | protein | F:receptor activity; F:protein binding;P:signal transduction; C:cytoskeleton | NP_567504 | AT4G16520 | ATG8F | ATG8F__Ubiquitin-like superfamily protein |
| M80608 | A_96_P143586 | 2,00 | down | Err:509 | P:response to stress; C:vacuole;P:carbohydrate metabolic process; P:cellular process | NP_193361 | AT4G16260 | | catalytic/ cation binding / hydrolase |
| DB727290 | A_96_P246190 | 2,00 | down | aaa-type atpase family protein | C:mitochondrion; F:nucleotide binding | NP_567238 | AT4G02480 | | AAA-type ATPase family protein |