UNIVERSITA' CATTOLICA DEL SACRO CUORE
PIACENZA

Scuola di Dottorato per il Sistema Agro-alimentare

Doctoral School on the Agro-Food System

cycle XXIII

S.S.D: BIO/04

Maize transcriptome analysis upon *Fusarium* infection in relation with host and pathogen genotypes.

Candidate: Alessandra Lanubile
Matr. n.: 3611478

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Chapter 1

Introduction

1.1 Plant - pathogen interactions

Plants have to defend continuously themselves from thousands of infectious diseases caused by a vast array of phytopathogenic fungi, bacteria, viruses, and nematodes. Only a small proportion of pathogens successfully invade the plant host and cause disease (Baker *et al.*, 1997). Pathogen failure depends on three reasons: 1) the plant is unable to support the requirements of a potential pathogen and this represents a nonhost; 2) the plant has preformed structural barriers or toxic compounds that limit successful infections to specific pathogen species; 3) upon recognition of the attacking pathogen, defense mechanisms are elaborated and infection remains localized. These three types of interaction are considered incompatible, but only the mechanism of resistance depends on induced responses. When the plant defenses are inadequate, the pathogen is not detected by the plant or the activated defense responses do not produce effects, successful pathogen invasion and disease (compatibility) happens (Hammond-Kosack and Jones, 1996).

One of the most common form of resistance shown by every species of plant against hundreds of potential pathogens is “non host resistance”. This resistance occurs in all genotypes of a plant species to all genotypes of a pathogen species (Rients and Thierry, 2009). This kind of resistance prescindes from the specific recognition of pathogen agent and seems to depend on a complex genetic control, that involves a multiplicity of unspecific defense factors, both constitutive and induced, considered as components of plant “basal resistence”. Simple mutagenesis, serial mutagenesis, gene silencing and gene expression studies, mostly in *Arabidopsis*, but also in barley, have led to the identification of genes and gene networks that contribute to nonhost and/or basal resistance, or act as negative regulators of defense reactions (Rients and Thierry, 2009). Key genes implicated in these processes are very diverse, and gene expression profiles indicate a large overlap of plant responses towards different microbe-associated molecular patterns (MAMPs), molecular sequences or structures in any pathogen-derived molecule perceived via direct interaction with host defense receptor (Mackey and McFall, 2006).

Non host resistance is different from “host resistance”, that is expressed by only one or few plant species, normally susceptible to a pathogen species, able to establish basic compatibility. In this case plant recognizes pathogen agent and activates a series of defense mechanisms that led to resistance. Contemporaneously to genetic

resistance, upon the interaction with the pathogen entity, plants can put in action many different biochemical and structural mechanisms, capables to contrast the pathological process in existence (post-infection resistance). Host resistance is often controlled by a single gene of resistance (*R*), whose product acts directly or indirectly with a specific pathogen elicitor, coded by a gene named of avirulence (*Avr*). In this case the interaction between the host (resistant) and the pathogen (virulent) is incompatible. If *R* and/or *Avr* genes are not present or functional, the host (resistant) - pathogen (avirulent) interaction is compatible and determines the development of disease. Host resistance was explained by Flor (1955) in the theory gene-for-gene, according to which in incompatible interactions for each gene that confers virulence (pathogenicity) to pathogen a correspondent gene of resistance is present in host plant, leading to pathogen resistance. The interaction between a dominant or semidominant resistance (*R*) gene product in the plant and a corresponding dominant phytopathogen avirulence (*Avr*) gene product is the cause of the activation of host defense mechanisms (Figure 1.1). It has been predicted that phytopathogen *Avr* products function as ligands and host *R* products function as receptors in an interaction leading to plant resistance to disease (Gabriel and Rolfe, 1990).

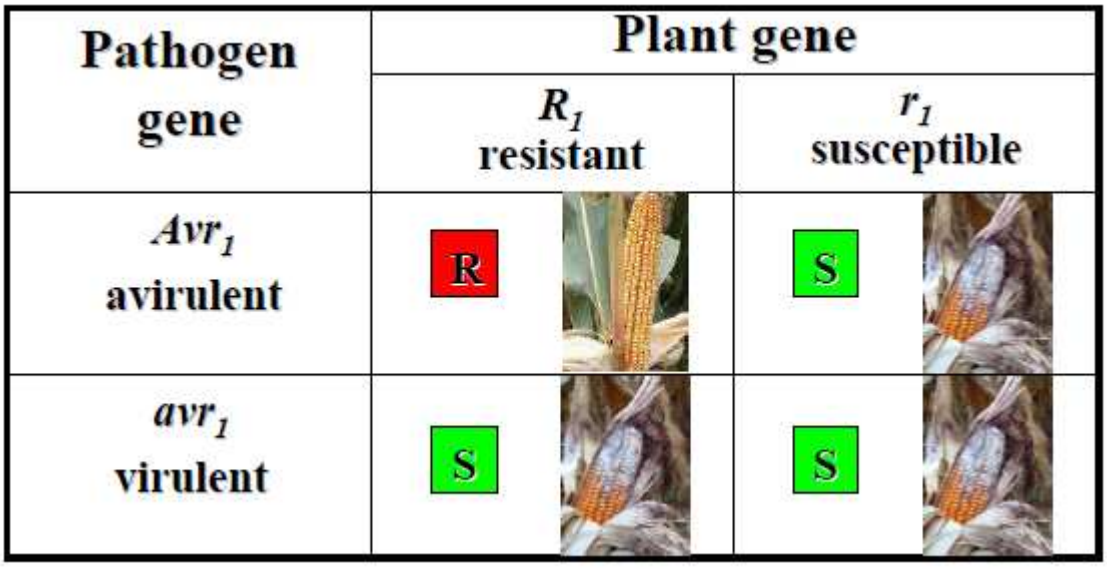


Fig.1.1 Interaction "gene for gene": *R* resistance, dominant allele, and *Avr* avirulence allele; R and S, host resistance and susceptibility, respectively.

1.2 Defense mechanisms

Plant defense mechanisms can answer both to many pathogens attacks and elicitors. The main mechanisms are: hypersensitive response (HR), systemic acquired resistance (SAR) and induced systemic resistance (ISR).

1.2.1 Hypersensitive response

The HR results in localized cell and tissue death at the site of infection, which constrains further spread of the infection (Dixon *et al.*, 1994) (Fig. 1.2). Classically, HR is defined as the death of host cells within a few hours of pathogen contact (Agrios, 1988), but the HR can be phenotypically diverse, ranging from HR in a single cell to spreading necrotic areas accompanying limited pathogen colonization (Holub *et al.*, 1994).

Fig. 1.2 A strong HR in maize leaves infiltrated with *Xanthomonas oryzae* pv. *oryzicola* 48 h before photography (Zhao *et al.*, 2005).

The HR has been suggested to play a causal role in disease resistance (Heath, 1980). In interactions involving obligate biotrophic pathogens that lead to the formation of haustorial associations with host cells, plant cell death would abstain the pathogen from the access to further nutrients. In hemibiotrophic and necrotrophic pathogens interactions, it is not clear the action of HR, because these pathogens can obtain nutrients from dead plant cells. In any case harmful preformed substances, stored in the vacuole, could be released by cellular decompartimentalization (Osbourn, 1996); or the concentrations of induced phytoalexins, normally metabolized in plant cells, may accumulate to inhibitory levels, because they are not longer turned over. In the biotrophic colonizations the HR can be mediated by messengers as salicylic acid (SA), and in the necrotrophic ones by jasmonic acid (JA) and ethylene (Van Wees *et al.*, 2000). The HR allows plants to recognize and resist to many invading phytopathogens, but it may also be a consequence of the activation of other defense mechanisms. Sometimes HR seems not to be involved in different gene-mediated

resistances, as the case of barley resistance against all races of *Erysiphe graminis* sp. *hordei*, which is conferred by the *mlo* gene (Freialdenhoven *et al.*, 1996) and the potato *Rx* gene-mediated resistance to potato virus X (Kohm *et al.*, 1993). The presence of the HR does not demonstrate that a resistance mechanism is activated. It is possible that the responses initiated by all *R* genes could result in HR, but some of them may hinder the disease so effectively that cell death is not activated. The HR often triggers nonspecific resistance throughout the plant, a phenomenon known as SAR (Figure 1.3) (Ryals J.A. *et al.*, 1996). Once triggered, SAR provides resistance to a wide range of pathogens for days. The HR and SAR depend on interaction between a dominant or semidominant resistance (*R*) gene product in the plant and a corresponding dominant phytopathogen avirulence (*Avr*) gene product, as predicted by Flor. Other aspects of the defense responses include an oxidative burst leading to production of reactive oxygen intermediates (ROIs), alteration of membrane potentials, cell wall modifications, lignin deposition, expression of defense related genes, an increase in lipoxygenase activity, and production of antimicrobial compounds, such as phytoalexins.

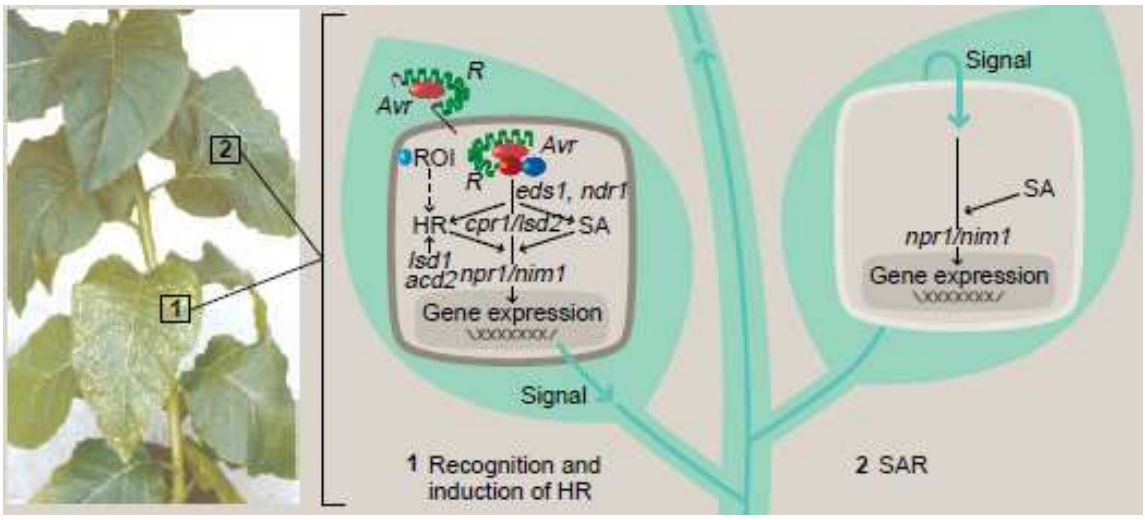


Fig. 1.3 The plant *R-Avr* gene interaction triggers a signal transduction pathway leading to the HR (1) and SAR (2). ROIs (reactive oxygen intermediates) may play a key signaling role in the induction of the HR. SA (salicylic acid) is involved in local (1) and systemic (2) resistance responses (Baker *et al.*, 1997).

1.2.2 Systemic acquired resistance

SAR refers to a distinct signal transduction pathway that plays an important role in the ability of plants to defend themselves against pathogens. The SAR pathway is activated after the formation of a necrotic lesion, either as a part of the HR or as a

symptom of disease. The development of a broad-spectrum, systemic resistance is the result of SAR activation (Hunt and Ryals, 1996; Neuenschwander *et al.*, 1996).

The SAR signal transduction pathway appears to function as a potentiator or modulator of other disease resistance mechanisms. When SAR is activated, a normally compatible plant-pathogen interaction (i.e., one in which disease is the normal outcome) can be converted into an incompatible one. Conversely, when the SAR pathway is incapacitated, a normally incompatible interaction becomes compatible (Mauch-Mani and Slusarenko, 1996). The mechanism by which this modulation occurs is not understood; however, at least part of the resistance response could be due to expression of the *SAR* genes.

SAR can be distinguished from other disease resistance responses by both the spectrum of pathogen protection and the associated changes in gene expression. In fact the expression of a set of genes called SAR genes is associated with SAR (Ward *et al.*, 1991). A SAR protein is a protein whose presence or activity correlates tightly with maintenance of the resistance state. Analysis of SAR proteins showed that many belong to the class of pathogenesis-related (PR) proteins, which originally were identified as novel proteins accumulating after tobacco mosaic virus (TMV) infection of tobacco leaves (Van Loon, 1985).

A number of viral, bacterial, and fungal pathogens can trigger both the expression of marker genes for SAR and the activation of SAR in a variety of dicotyledons, although the identity and relative expression levels of *SAR* genes vary between different plant species. Such species-specific differences may reflect different evolutionary or breeding constraints, that have selected for the most effective SAR response against the particular suite of pathogens to which an individual species is subject. Also in monocotyledon species a number of genes homologous with *SAR* genes from dicotyledons were identified. Homologs of the PR-1 family have been characterized in maize and barley, and additional PR proteins have been identified in maize (Nasser *et al.*, 1988).

SAR is also associated with the high-enhanced production of salicylic acid. SA is required for *SAR* and *PR* gene expression. Whether SA acts as a longdistance systemic signal for SAR induction in plants remains unclear. Many studies have reported the strong correlation between the concentration of SA and the establishment of enhanced disease resistance in numerous plants (Malamy *et al.*, 1990; Cameron *et al.*, 1994). For example the analysis of transgenic plants expressing the bacterial *nahG* gene encoding salicylate hydroxylase, an enzyme that

catalyzes the conversion of SA to catechol, supports this theory. In fact these plants are not only unable to accumulate free SA, but they are incapable of mounting a SAR response to viral, fungal, or bacterial pathogens (Lawton *et al.*, 1995), indicating that SA accumulation is required for SAR induction.

1.2.3 Induced systemic resistance

ISR of plants is a widespread phenomenon used in plant protection. The resistance induced is characterized by a restriction of pathogen growth and a suppression of disease symptom development (e.g. a reduction in lesion size and/or number) compared with non-induced plants infected with the same pathogen (Hammerschmidt, 1999).

ISR develops as result of colonization of plants roots by plant growth-promoting rhizobacteria (PGPR) and is mediated by a SA-indipendent pathway. It was demonstrated that ISR acts indipendently from SA and activation of PR genes, requiring only JA and ethylene. The expression of known defense-related genes is not associated to ISR, but upon challenge with a pathogen, plants expressing ISR exhibit an enhanced expression of certain JA-responsive genes (Pieterse and Van Loon, 2007).

The event called “priming” is characteristic of induced resistance. This mechanism is not directly activated by the inducing agent, but instead are potentiated for enhanced expression upon subsequent pathogen attack.

Induced resistance is a complex plant response to pathogen attack and as such, may be modified by many factors including genotype and environment (Walters, 2009).

1.2.4 Signaling mechanisms in defense response

Plants can respond to pathogens invasion through a complex activation of defense strategies (Bowles, 1990; Dixon *et al.*, 1994; Zhu *et al.*, 1996).

Figure 1.4 shows an overview of signal transduction pathways involved in the activation and coordination of the local induction of plant defense responses. Plant proteic receptors (Rp) intercept signals deriving from pathogen or dipendent from interactions. These signals include both direct or indirect products of *Avr* genes, physical contacts and general components of each organism such as chitin, enzymes and cell wall fragments of plants. Plant “Rp” can be coded or not by *R* genes. The events that are immediately downstream of signaling transmission involve kinases, phosphatases, G proteins and ion fluxes. These events produce and rapidly activate

numerous and different compounds, like ROIs and nitrogen oxyde (Delledonne *et al.*, 2001), and lead to the direct induction of the transcription of defense genes (or possibly apoptosis genes). The amplification of the initial defense response occurs through the production of additional molecular signals, such as other ROIs, peroxide lipids, benzoic acid (BA), JA biosynthesis and ethylene. These in turn activate other defense genes and modify defense proteins and enzymes. Concomitant alterations to cellular redox status and/or cellular damage induce preformed cell protection mechanisms (that is, the Halliwell-Asada cycle, plastid-localized SODs, and catalases) and genes encoding various cell protectants. Defense-related stress may also induce apoptosis. Cross-talk between the various induced pathways coordinate the responses.

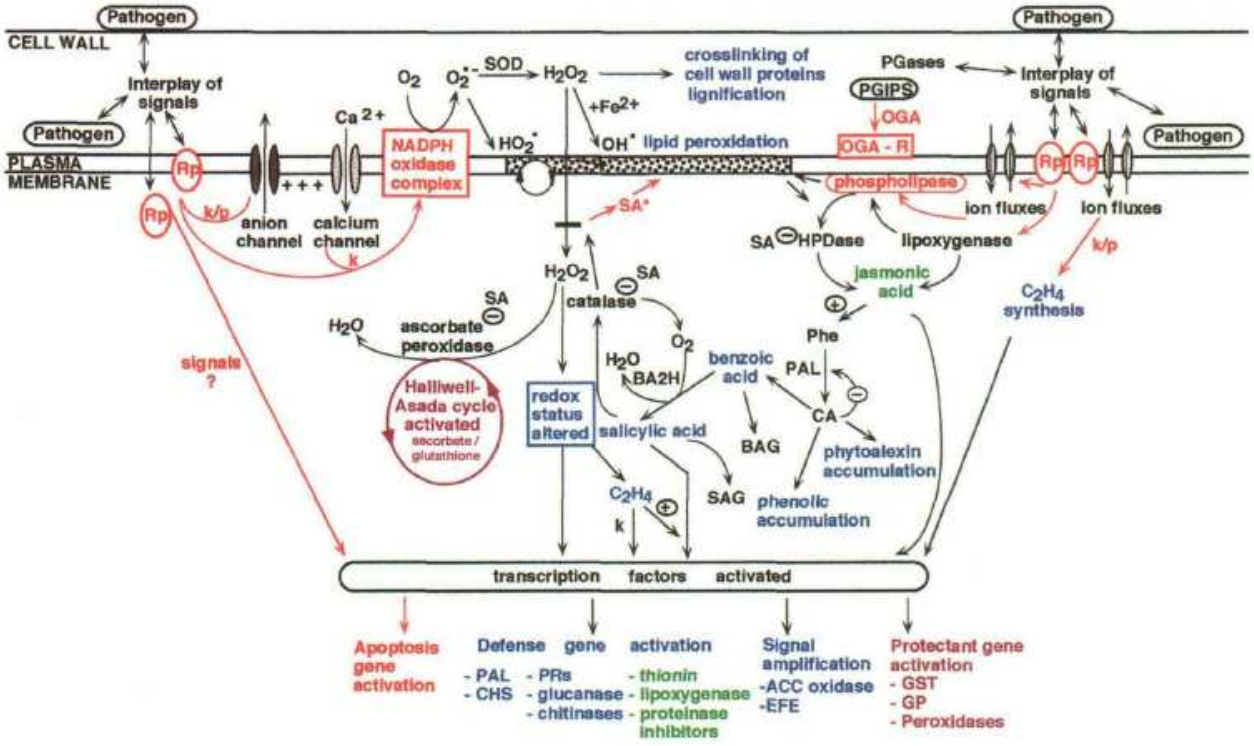


Fig. 1.4 Overview of signal transmission pathways involved in plant defense response (modified from Hammond-Kosack and Jones, 1996). (+) indicates positive and (-) indicates negative interactions. Components and arrows indicated in red are only postulated to be present in plant cells, whereas those in blue indicate known plant defense responses; green indicates plant defense responses also activated by JA, and purple indicates plant protection mechanisms. ACC oxidase, 1-aminocyclopropane-1-carboxylate oxidase; BAG, benzole acid glucoside; BA-2H, benzole acid-2 hydroxylase; CA, cinnamic acid; CHS, chalcone synthase; EFE, ethylene-forming enzyme; HO_2, hydroperoxyl radical; HPDase, hydroxyperoxide dehydrase; GP, glutathione peroxidase; GST, glutathione S-transferase; k, kinase; O_2^-, superoxide anion; OH^- , hydroxyl radical; OGA and OGA-R, oligalacturonide fragments and receptor; p, phosphatase; PAL, phenylalanine ammonia-lyase; PGases, polygalacturonases; PGIPS, plant polygalacturonic acid inhibitor proteins; Phe, phenylalanine; PR, pathogenesis-related; Rp, plant receptor protein; SA and SAG, salicylic acid and salicylic acid glucoside; SA^-, SA radical; and SOD, superoxide dismutase.

1.2.4.1 Reactive oxygen species

ROIs are key signal in plant defense. Generation of ROIs, which in some cases requires activation of Ca^{++} and anion channels, occurs within minutes after *R-avr* interactions. ROIs may directly trigger the HR or cell death and the subsequent induction of defense-related genes.

ROIs generation and defense responses can be blocked by diphenylene iodonium, an inhibitor of mammalian NADPH oxidase, which suggests that a similar system is required in plants. Furthermore, antibodies to various mammalian NADPH oxidase components cross-react with plant proteins of similar size and the *rbohA* gene

product from rice has been found as homolog of the gp91phox component of NADPH oxidase (Baker *et al.*, 1997).

The presence of superoxide anions (O_2^-) was reported for the first time by Doke and colleagues (1988), that detected the production of O_2^- in incompatible interactions, initially between potato and *Phytophthora infestant* (late blight fungus) and then between tobacco and TMV. The O_2^- generated is usually rapidly dismutated either non enzymatically or via superoxide dismutase (SOD) catalysis to hydrogen peroxide (H_2O_2; Figure 1.5). Both O_2^- and H_2O_2 can cross biological membranes and cause cellular damage.

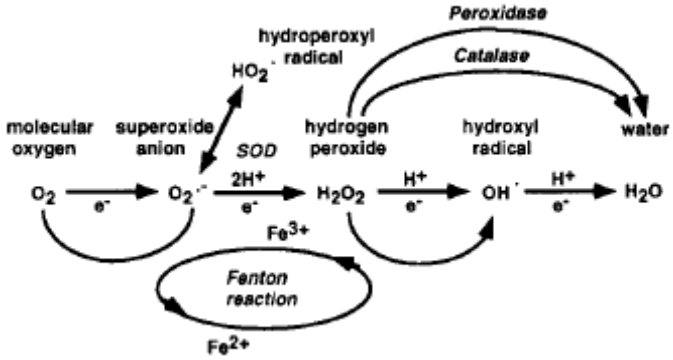


Fig. 1.5 Interconversion of ROIs derived from molecular oxygen (Hammond-Kosack and Jones, 1996).

In the presence of Fe^{++}, H_2O_2 can undergo the Fenton reaction that gives rise to the extremely destructive hydroxyl free radical (OH^-), which can initiate self-perpetuating lipid peroxidation.

ROIs present several roles in plant defense. H_2O_2 is toxic for microbes and can contribute to the structural reinforcement of plant cell walls. It is also essential for the formation of lignin polymer precursors via peroxidase activity and increases benzoic acid-2 hydroxylase (BA2-H) enzyme activity (Léon *et al.*, 1995), which is required for SA biosynthesis. In addition H_2O_2 activates some protection mechanisms, such as glutathione S-transferase gene expression, in neighboring cells (Levine *et al.*, 1994) or the synthesis of antimicrobial phytoalexins, through the activation of the phenylpropanoid or flavonoid biosynthetic pathways.

The stability of specific defense-related mRNA transcripts may be also regulated by redox balance in plants (Mehdy, 1994).

1.2.4.2 Benzoic acid and salicylic acid

Both BA and SA and their glucoside conjugates are accumulated at high concentrations in the immediate vicinity of the incompatibles infection sites.

The two compounds are commonly associated with HR. BA and SA derive from the phenylpropanoid pathway and have several roles in the plant defense response.

The presence of SA in some incompatible interactions is absolutely necessary, as showed in transgenic tobacco and *Arabidopsis* lines that expressed a bacterial *nahG* gene, encoding the enzyme salicylate hydroxylase. Salicylate hydroxylase converts SA to catechol, and these transgenic plants have markedly reduced levels of SA. The lack of SA accumulation in these nahG-expressing plants correlated with weakened local *R* gene-mediated resistance responses and also with a block in the induction of various defense genes (Ryals *et al*., 1996).

1.2.4.3 Pathogenesis-related proteins and lipoxygenases

The PR proteins are definied as proteins coded by host plant, but induced specifically in pathological or related situations. They do not only accumulate locally in the infected leaf, but they are also induced systemically, associated with SAR against infection caused by fungi, bacteria and viruses. Originally five main classes of PRs (PR1-5) were characterized by both biochemical and molecular-biological techniques in susceptible tobacco genotypes in response to TMV infection. In the past ten years, many PRs have been identified and classified into 17 families (PR1 to PR17) based on their differential structures and biological activities (Eulgem *et al.*, 2005). PR proteins are also present in monocotyledons plants. White and colleagues (1999), using immunoelectroblotting technique, have shown the presence of tobacco PR1-type proteins in mildew-infected barley and in brome mosaic virus (BMV)-infected maize. In addition eight proteins induced in maize leaves upon mercuric chloride treatment or BMV infection have been identified by Nasser *et al.* (1988). Several PR proteins possess either antifungal or antibacterial activity in vitro and are now known to be chitinases, glucanases, or to bind chitin. The degradation of fungal cell wall structural polysaccharides, or the alteration of fungal cell wall architecture, could arrest or severely impair fungal growth. Moreover, the constitutive expression of PR proteins of known and unknown function in transgenic plants has led to increased resistance to some fungal pathogens.

The basic cysteine-rich thionins, found mainly in cereals, are another group of defense-related proteins with known antimicrobial activity. Like the PR proteins, these thionins also accumulate differentially during incompatible interactions. Interestingly, a JA-mediated signal transduction pathway, distinct from the typical SA-mediated pathway leading to PR gene activation, controls thionin gene expression in *Arabidopsis* (Epple *et al.*, 1995).

Associated with *R-Avr* gene mediated incompatibility is the rapid increases in lipoxygenase (LOX) enzyme activity and/or mRNA and protein levels. These enzymes can contribute to resistance in a number of ways. For example, LOX may generate signal molecules such as JA, methyl-JA, or lipid peroxides, which coordinately amplify specific responses. Irreversible membrane damage may also be caused by LOX activity, which would lead to the leakage of cellular contents and ultimately results in plant cell death. Alternatively, LOX-catalyzed reactions can result in the production of toxic volatile and nonvolatile fatty acid-derived secondary metabolites that could directly attack invading pathogens (Croft *et al.*, 1993).

1.2.4.4 Phytoalexins

The phenylpropanoid pathway leads to biosynthesis of a wide variety of phenolic compounds, that have resistance or tolerance against abiotic and biotic factors. For example, induced expression and accumulation of flavonoid and isoflavonoid phytoalexins has been shown to impart resistance against fungal and bacterial diseases. Phytoalexins are low molecular weight, lipophilic, antimicrobial compounds that accumulate rapidly around sites of incompatible pathogen infections and in response to an extensive array of biotic and abiotic elicitors. In maize, upon fungal inoculation, induction of 3-deoxyanthocyanidins was observed in silks and kernels of resistant lines, suggesting the role of these compounds in resistance to *F. verticillioide*s (Sekhon *et al.*, 2006).

In *Arabidopsis*, camalexin accumulates in response to both virulent and avirulent bacterial pathogens. In plants with phytoalexin-deficient (*pad*) mutations (Ausubel, *et al.*, 1995), growth of the avirulent bacteria was not compromised and expression of *SAR* genes was not affected. Thus, phytoalexins may not play a significant role in gene-for gene–type resistance. However, the *pad1* and *pad2* mutants were more susceptible to virulent bacteria than were wild-type plants.

1.2.4.5 Cell wall proteins

The fortification of the plant cell wall can increase disease resistance in various ways. For example the formation of papillae (Figure 1.6) occurs rapidly in response to fungal invasion upon the penetration peg and they physically block fungal penetration of host cells (Bayles *et al.*, 1990). However, it is also possible that their formation is required to provide adequate support for subsequent haustorium development, in which case they may be essential for pathogenesis.

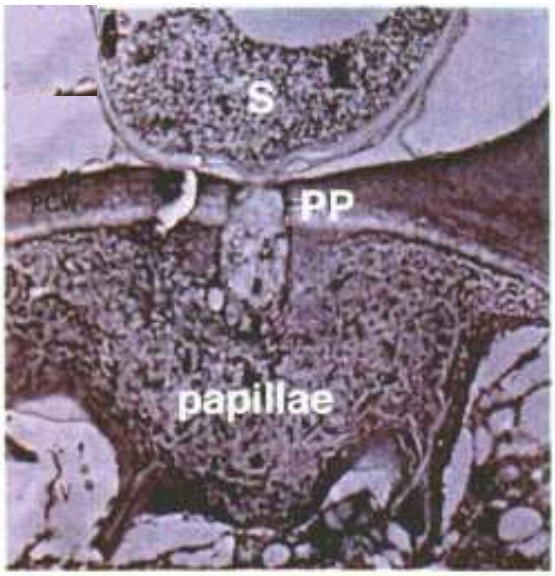


Fig.1.6 Papillae formation. PP, penetration peg; S, germinating spore. (Hammond-Kosack and Jones, 1996).

The expression of polygalacturonase-inhibiting proteins (PGIPs) inhibit polygalacturonases (PGs), pathogen enzymes that degrade the various plant cell wall polymers. PGlPs may retard PG function or alternatively may slow the rate of hyphal extension so that other components of the defense response can be more effectively deployed.

Rapid callose deposition in cell walls is also frequently associated with sites of pathogen incompatibility. Callose deposition also occurs when plant cell cultures are challenged with pathogen-derived elicitors or when plant tissue is mechanically wounded. Therefore callose deposition induction blocks plasmodesmata, preventing cell-to-cell movement of viruses (Parker *et al.*, 1993; Beffa *et al.*, 1996).

The basic hydroxyproline-rich glycoproteins (HRGPs) play an important role in the organization of primary cell wall architecture and may act as the foci for the initiation of lignin polymerization. The local elevation of lignin content is an additional but probably slower mechanism that renders cell walls more

impermeable. In addition, extensins are capable of immobilizing certain plant pathogens through electrostatic interactions (Showalter, 1993).

1.3 The genus *Fusarium*

Fusarium species are filamentous Ascomycete fungi widely distributed in soil and plants. The genus *Fusarium* contains over 20 species and includes several economically important plant pathogens, most notably *F. verticillioides, F. graminearum,* and *F. oxysporum*. Studies on *Fusarium* species have been very active lately, mostly due to the concerns regarding various mycotoxins in crops infected with toxigenic *Fusarium* (Desjardins, 2006). The three forementioned *Fusarium* species are responsible for the significant economic loss by *Fusarium*-caused plant diseases worldwide (McMullen *et al.*, 1997; Munkvold and Desjardins, 1997). *Fusarium* species have distinct climatic preferences. The climate, and even local variation in weather, can limit the series of species observed. There are species that prefer tropical climates, hot arid climates, or temperate climates (Summerell *et al.*, 2003). The distribution and the prevalence of different *Fusarium* species, depending on different environmental conditions, determine two kinds of ear rot disease in maize ears and kernels (Arino and Bullerman, 1994).

Gibberella ear rot or "red ear rot" usually initiates from tip of the ear and develops a red or pink mould covering a large proportion of the ear. Usually, it is caused by *F. graminearum* (Bechtel *et al.*, 1985), although in Europe several other *Fusarium* species may be associated with this disease, especially *F. culmorum* (Logrieco *et al*., 2002). *Gibberella* ear rot predominates in cooler areas or those with higher precipitation during the growing season (Bechtel *et al.*, 1985; Logrieco *et al.*, 1993). *Fusarium* ear rot typically occurs on random groups of kernels or on physically injured kernels (Miller, 1995) and consists of a white or light pink mould. Light pink fusariosis prevails in drier and warmer climates of Southern Europe areas (Bottalico and Logrieco, 1988). Identical symptoms are caused by *F. verticillioides, F. proliferatum* or *F. subglutinans,* but occasionally other *Fusarium* species are associated with these symptoms. Historically *F. verticillioides* has been reported as the most common pathogen causing *Fusarium* ear rot (Bottalico, 1998).

1.3.1 The disease caused by *F. verticillioides*

Fusarium verticillioides (Sacc.) Nirenburg (teleomorph *Gibberella moniliformis* Wineland) causes severe stalk rot and ear rot of maize and is found in plant residues in almost every maize field at harvest (White, 1999). In the Southern regions of Europe *F. verticillioides* is the prevaling species in maize fields (Logrieco *et al.*, 2002; Folcher *et al.*, 2009). *F. verticillioides* is associated with disease at all stages of corn plant development infecting roots, stalks and kernels. Therefore symptomless infection can exist throughout the plant in leaves, stems, roots, grains, and the presence of the fungus is in many cases ignored because it does not cause visible damage to the plant (Battilani *et al.*, 2003). Fungal stalk rots, including fusarium stalk rot, are the most devastating diseases of maize in terms of average yield loss. *F. verticillioides* ear rot is more severe when hot, dry weather occurs. Symptoms vary greatly depending upon genotype, environment and disease severity. Usually, individual or groups of infected kernels are scattered randomly on the entire ear. Whitish pink to lavender fungal growth on kernels and/or silks is typical. Infected kernels may also exhibit a "starburst" symptom, i.e., white streaks radiating from the point of silk attachment at the cap of the kernel or from the base (Figure 1.7).

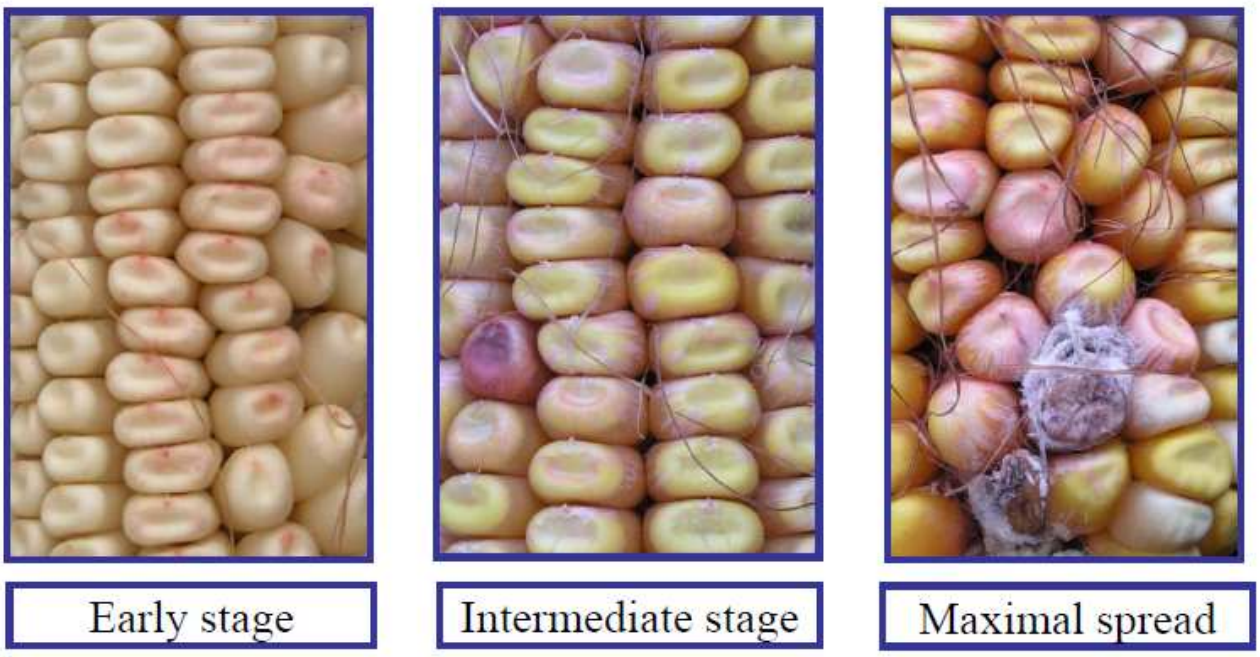


Fig. 1.7 Stages of infection by *Fusarium verticillioides* in maize kernels.

F. verticillioides can enter a maize plant systemically from the seed (Oren *et al.*, 2003), through wounds in the plant or through infections of the silks. Of these

different routes, kernel infection occurs most efficiently from strains that are inoculated into the silks (Munkvold *et al.*, 1997). Disrupting husk integrity increase ear rot severity and drought stress increases the amount of stalk rot and can be relieved by irrigation.

1.3.2 Secondary metabolites biosynthesis in *Fusarium verticillioides*

Recently, attention to maize diseases caused by *F. verticillioides* has increased due to the fact that the fungus can produce a wide variety of secondary metabolites, which include fumonisins, bikaverin, fusaric acid, fusarin C, and moniliformin (Nelson *et al.*, 1993; Bacon *et al.*, 2004). Of these secondary metabolites, fumonisins have been reported to possess a high cancer promoting activity (Gelderblom *et al.*, 1988). The ingestion of fumonisin-contaminated corn by humans and animals has been linked to a variety of illnesses, including leukoencephalomalasia and neural tube defects (Gelderblom *et al.*, 1988; Marasas, 2001; Minorsky, 2002; Missmer *et al.*, 2006). More than 28 fumonisin analogs have been identified to date, and among them, fumonisin B_1 (FB_1) is the major fumonisin found in contaminated commodities (Rheeder *et al.*, 2002). To date, efficient management strategies against *F. verticillioides* have not been developed (Munkvold and Desjardins, 1997). Moreover, the chemical structure of FB_1 is so stable that it cannot be completely eliminated by post-harvest treatment (Munkvold and Desjardins, 1997).

Over the past decade, significant progress has been made in elucidating the molecular genetic mechanisms associated with fumonisin biosynthesis in *F. verticillioides*. FB_1 is synthesized via a polyketide biosynthetic pathway as shown in Figure 1.8 (Butchko *et al.*, 2006).

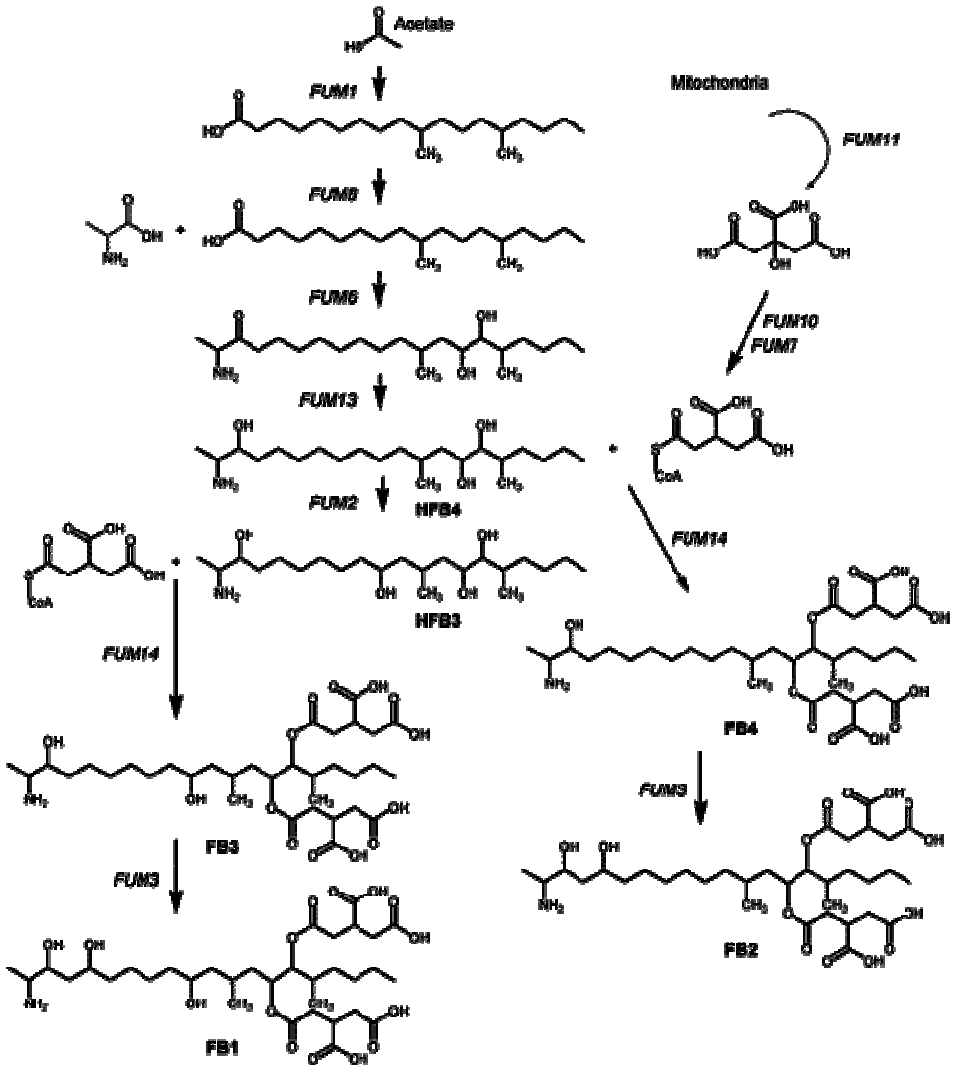


Fig.1.8 Proposed fumonisin biosynthetic pathway (Butchko, 2006).

The genes involved in FB_1 biosynthesis are clustered in a fashion similar to other well-defined fungal secondary metabolite gene clusters. The fumonisin (*FUM*) gene cluster is known to contain 22 genes with a length of 42 kb (Brown *et al.*, 1996; Seo *et al.*, 2001; Proctor *et al.*, 2003; Sagaram *et al.*, 2006b). Of the 22 genes, 15 genes are coregulated including the key gene in the *FUM* gene cluster, *FUM*1, which encodes a polyketide synthase (Proctor *et al.*, 1999). The FB_1 structure suggests that at least 8 enzymatic steps are required for its synthesis (Seo *et al.*, 2001). *In silico* analysis of the *FUM* cluster suggest that the Fum1 protein is involved in the formation of a linear polyketide. An aminotransferase, the Fum8 protein, attaches an alanine to the polyketide backbone and P450 monoxygenase, the Fum6 protein, catalyzes further modification of side chains. Gene disruption of *FUM*1, *FUM*6, and *FUM*8 demonstrated that these genes are indispensable for FB_1 biosynthesis (Seo *et al.*, 2001; Proctor *et al.*, 2003). Biochemical functions of the Fum3 protein, a 2-

ketoglutarate dependent dioxygenase, and the Fum13 protein, a dehydrogenase/reductase, were characterized recently (Butchko *et al.*, 2003; Ding *et al.*, 2004). Genes for self-protection, such as longevity assurance factor (*FUM*17 and *FUM*18) and an ABC transporter (*FUM*19), are also present in the *FUM* cluster (Proctor *et al.*, 2003).

While our knowledge of the *FUM* gene cluster is nearly complete, we still lack a clear understanding of the regulatory mechanisms involved in fumonisin biosynthesis. Therefore, recent research has been mostly directed at the regulation of FB_1 biosynthesis. Interestingly, specific regulatory gene(s) that are often present in fungal secondary metabolite gene clusters have not been reported in the *FUM* gene cluster. Environmental factors, mainly acidic pH and nitrogen stress, have been shown to enhance FB_1 production (Shim and Woloshuk, 1999). In addition, maize kernel composition was identified as a factor in FB_1 biosynthesis (Bluhm and Woloshuk, 2005; Shim *et al.*, 2003). Among the regulatory genes associated with FB_1 biosynthesis, *FCC*1 was the first regulatory gene characterized in *F. verticillioides* (Shim and Woloshuk, 2001). *FCC*1 encodes a C-type cyclin, and the *FCC1* knock-out mutant (FT536) failed to produce FB_1 on corn kernels. In addition, FT536 showed significant reduction in conidia production in synthetic media (Shim and Woloshuk, 2001). Based on this phenotypic analysis, it was suggested that *FCC*1 plays an important role in fumonisin biosynthesis and conidiation in *F. verticillioides*. Since *FCC*1 is associated with multiple signaling pathways, it has been emphasized that identification of additional downstream signaling genes is necessary to better understand the *FCC*1-mediated signaling pathway that directly impacts FB_1 biosynthesis. However, we still have limited knowledge of downstream genes of *FCC*1. To date, studies have only revealed a limited number of regulatory genes associated with fumonisin biosynthesis, e.g., *PAC*1, *FCC*1, *ZFR*1, *GBP*1, and *FUM*21 (Shim and Woloshuk, 2001; Flaherty *et al.*, 2003; Flaherty and Woloshuk, 2004; Sagaram *et al.*, 2006a; Brown *et al.*, 2007). *GBP*1 and *PAC*1 are negative regulatory genes, while *FCC*1 and *ZFR*1 positively regulate FB_1 biosynthesis. Two genes, *ZFR*1 and *GBP*1, were determined as *FCC*1-downstream genes acting on FB1 biosynthesis (Flaherty and Woloshuk, 2004; Sagaram *et al.*, 2006a). *FUM*21 is a Zn(II)-2Cys6 DNA binding transcription factor, that positively regulates *FUM* gene expression and is required for fumonisin synthesis (Brown *et al.*, 2007). Significantly, other than *FUM*21, none of these genes are physically linked to the

FUM gene cluster, and we have limited understanding of fumonisin regulatory signaling network in *F. verticillioides*.

F. verticillioides secondary metabolites are not limited to fumonisins. There are a variety of other mycotoxins (e.g, fusaric acid) and pigments (Nelson *et al.*, 1993; Bacon *et al.*, 2004;) synthesized by the fungus. While studies have begun revealing the regulatory mechanism associated with FB_1 biosynthesis, little is known about the biosynthesis of other *F. verticillioides* secondary metabolites, such as bikaverin, fusarin C and moniliformin. The genes and signaling pathways associated with biosynthesis of these metabolites are not fully understood. Moreover, it is important to recognize that secondary metabolism in *F. verticillioides* is complex and that these biosynthetic processes are interlinked and intricately regulated.

1.3.3 Cytology of infection by *F. verticillioides* in maize

The hystological analysis of penetration and colonization by *F. verticillioides* in maize seedlings has allowed the citological inspection of the fungal development in the host (Murillo *et al*., 1999).

Results obtained by light microscopic observations of trypan blue-stained sections of germinating maize embryos have showed that fungal hyphae growing at the surface of the scutellum were clearly visible within 48 to 72 h after inoculation.

The process of infection by *F.verticillioides* in maize seedling tissues have been also studied through scanning electron microscopy (SEM), that has revealed fungal hyphae growing on the surface of seed pericarp and able to penetrate it. In germinating maize embryos fungal entrance into the scutellum was mainly intercellular, although direct penetration into parenchima cells also occured (Figure 1.9).

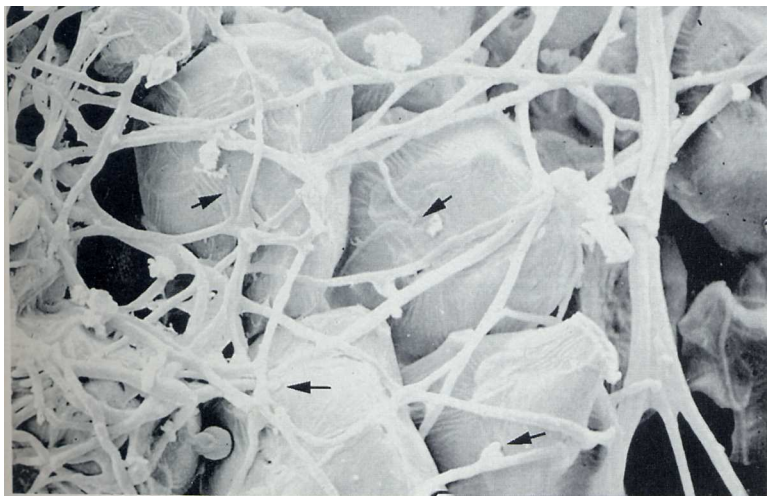


Fig. 1.9 *F.verticillioides* colonization of the scutellum parenchima cells. Arrows indicate the sites where the fungus penetrates into the host cell (Murillo *et al*., 1999).

Fungal colonization was also analyzed through transmission electron microscopy (TEM): it was possible to observe an amorphous matrix linking the surface of the epidermal cells and surronding fungal cells. The *F.verticillioides* cells were characterized by a dense cytoplasm with a thin cell wall attached to the host wall, often distorted at the contact sites. Furthermore it was observed the deposition of electron-opaque materials on the outer cell wall surface at the sites of fungal penetration and the formation of protuberances, known as papillae (Figure 1.10 A, B, C).

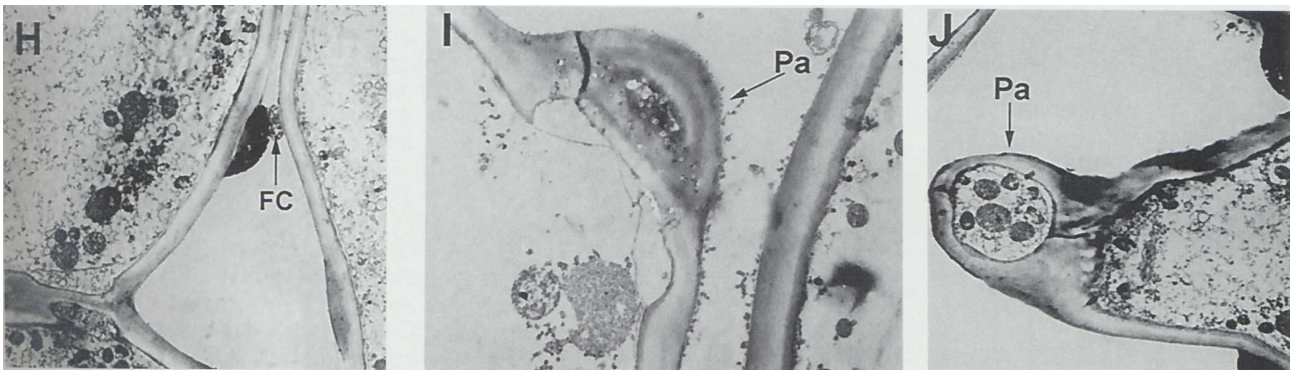


Fig. 1.10 A, B and C The fungus invades the scutellum via intercellular spaces (A). Formation of papillae (B,C). FC, fungal cell; Pa, papilla (Murillo *et al*., 1999).

In addition, cellular and subcellular immunolocalization studies have revealed the accumulation of pathogenesis-related maize seed proteins (PR) at very high levels in the aleurone layer of germinating seeds, in the scutellar epithelial cells of germinating embryos and within papillae.

1.3.4 Host genes providing resistance to *Fusarium* infection

Much progress has been made on *F. verticillioides* genomic resources, that have provided researchers with powerful tools to speed the process of identifyng new fungal genes and understanding their role in pathogenesis events and mycotoxin biosynthesis. On the contrary, the molecular interaction between the fungus and the plant is not well known and few informations are now availables about the defense response of maize against *F. verticillioides* infection.

The induction of specific PR proteins and protein-kinases following *F.verticillioides* infection have been reported in the maize inbred line W64A (Murillo *et al.*, 2001). A full-lenght cDNA coding for a calcium-dependent protein kinase (CPK) was identified in W64A maize inbred, resistant to *F. verticillioides*. Ca^{++}-regulated protein phosphorilation is a process often associated to the activation of the expression of pathogenesis-related genes. Calcium regulates the activity of these kinases, binding and activating the typical calcium-binding domains, essentials to mediate the plant response to different stimuli. The *ZmCPK*10 gene, encoding a specific maize kinase, was transcriptional activated in elicitor-treated or fungal-infected germinating maize seeds. A strong cell-type-specific expression of this gene was displayed through *in situ* mRNA hybridization in infected tissues and not in sterile tissues. Therefore the activation of the kinase was associated to a high level of the *PRms* mRNA (Murillo *et al.*, 2001). In a previous work, Murillo *et al.* (1997) showed that the *PR* maize genes had remarkable cell-type specific expression during growth of the *Fusarium*-infected maize radicle, specifically in the parenchima cells of the differentiating xylem elements, in the pericycle and during primordium development of the lateral root. *ZmCPK*10 gene expression occurred always in those specific cell types and developmental stages in which *PRms* expression was present. It will be necessary to demonstrate that CPK10 induces the *PRms* gene expression and the response of other genes involved in the plant defense (Murillo *et al.*, 2001).

In several plant-pathogen interactions, defense response has been associated with induction of phenylalanine ammonia lyase and chalcone synthase, and accumulation of flavonoids, phenolic compounds, and phytoalexins. Secondary metabolites in maize kernel are thought to play an important role in plant resistance against ear rot fungi. Sekhon *et al.* (2006) showed that the amount of phenolic and flavones present in the uninoculated silk tissue is not a good indicator of maize resistance to *Fusarium* ear rots. However, upon fungal inoculation, induction of 3-deoxyanthocyanidins was observed in resistant lines suggesting the role of these

compounds in resistance to *F. verticillioides.* A decrease in accumulation of 3-deoxyflavonoids in susceptible lines could be due to inhibition of the plant metabolism by the fungus. There did not appeared to be a direct correlation between the induced expression of flavonoid biosynthesis genes in the greenhouse grown plants and field based ear rot severity.

Specific genes putatively providing resistance to *Fusarium* pathogens have recently been identified. Gao and colleagues (2007) reported that a defective lipoxygenase mutation (*lox3*) in maize reduces fumonisin B1 contamination and *F. verticillioides* conidiation as well as providing resistance to several other pathogens of maize. In addition, Yuan and colleagues (2007) reported a maize guanylyl cyclase gene associated with resistance to *Gibberella* ear rot, caused by the related fungus *F. graminearum*. Two of the multiple copies of this gene in maize map near QTL positions for ear rot resistance. The gene transcript is up-regulated in response to pathogen inoculation, and the more resistant parent has a higher level of expression of the gene family. If markers can be developed to distinguish the resistant alleles consistently across breeding populations, then such markers would be ideal tools for implementing marker-assisted selection on a broader scale in maize breeding programs.

The maize *An*2 gene encodes a copalyl diphosphate synthase (CPS)-like protein with 60% amino acid sequence identity with the *An*1 gene product involved in gibberellin biosynthesis. The *An*2 transcript levels were strongly up-regulated by *Fusarium* attack, with an increase in silk, husk and ear tip tissues as early as six hours after inoculation of silk channels with spore suspensions of various *Fusarium* sp. (Harris *et al.*, 2005). The *Fusarium*-inducible nature of *An*2 is also consistent with a previous report that cell-free extracts from maize seedlings produce diterpenes in response to *Fusarium* infection. However, it is not known whether *An*2 is involved in defense-related secondary metabolism in addition to gibberellin synthesis.

Proteomic studies represent an alternative approach to evaluate the plant defense response to pathogen infection. Campo *et al.* (2004) performed experiments in germinating W64A maize embryos to identify the response to fungus infection at protein level. High-resolution two-dimensional gel electrophoresis system was used to identify the changes of plant defense proteins. Homology-based searching with MALDI-TOF data in association with nanospray ion-trap tandem mass spectrometry (nESI-IT MS/MS) allowed to detect different kinds of antioxidant enzymes, such as

Cu/Zn-superoxide dismutase, glutathione-S-transferase and catalase, that normally protect cells from oxidative damage.

Proteins involved in initiation of other protein synthesis or that partecipate in the protein folding process and stabilization were also identified in the infected embryos. The eukaryotic translation initiation factor 5A could be implicated in the translation of mRNAs involved in the plant defense response. In addition it was observed an up-regulation of the enzyme aldolase and a down-regulation of the glyceraldehyde 3-phosphate dehydrogenase, that suggested a sugar metabolism alteration in infected plants. Finally β-1,3-glucanases and chitinases resulted highly expressed through immunoblot analyses. These PR proteins are costitutively expressed, showing that their expression is not only linked to pathogen infection but also to the normal process of seed germination (Campo *et al.*, 2004).

1.3.5 Genetic and molecular approaches to reduce *Fusarium* ear rot and mycotoxins contamination

Genetic variation for resistance to *Fusarium* ear rot exists among inbred lines and hybrids of field maize (Clements and White, 2004; Eller *et al.*, 2008b). There is no evidence of complete resistance to either ear rot or fumonisin contamination in maize. Resistance to *Fusarium* in maize can be seen as consisting of two components: (1) resistance to initial penetration, and (2) resistance to the spreading of the pathogen in host tissue. Percentage of infected kernels is the result of resistance components 1 and 2. A screening trial was conducted for both *Fusarium* ear rot and fumonisin concentration using public and private inbreds (Berardo *et al.*, 2006). Each plant in this trial was inoculated with a mixture of two strains of *F. verticillioides* with the pin-bar method at 10 days post mildsilk. This screen has identified several lines with good levels of resistance to both *Fusarium* ear rot and fumonisin accumulation (Figure 1.11).

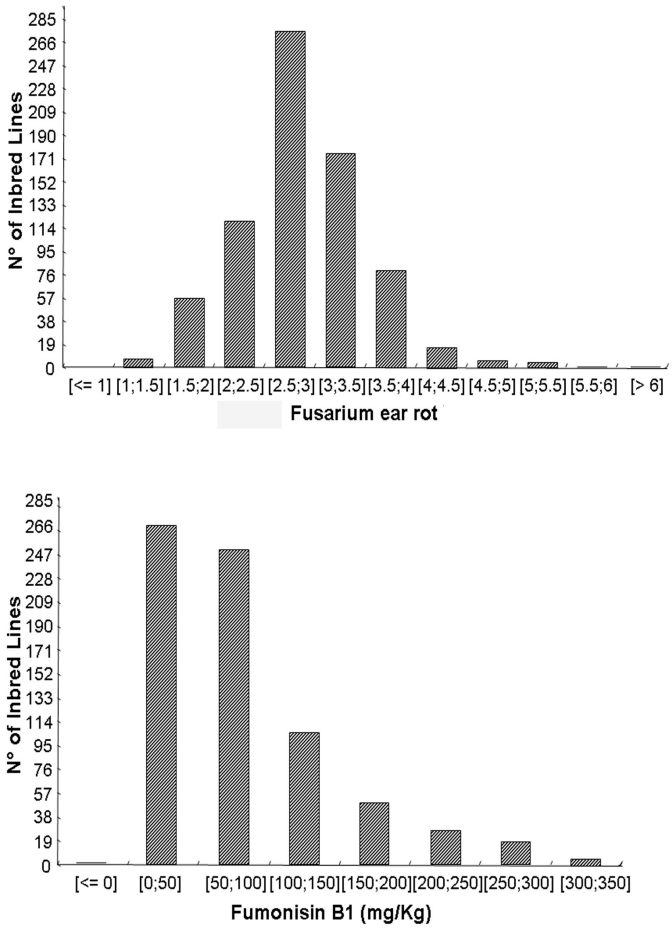


Fig.1.11 Distribution of *Fusarium* ear rot (top; rating 1 = 0%; 6 ≥ 80%.) and fumonisin B_1 content (bottom) among 728 maize inbred lines artificially inoculated with two strains of *F. verticillioides* at 10 days post mid-silk by injecting a spore suspension through the husk.

Perez-Brito *et al.* (2001) demonstrated that resistance to *Fusarium* ear rot in two F_2 tropical maize populations is polygenic with relatively low heritability (h^2 = 0.26-0.42). Clements *et al.* (2004) screened topcrosses of 1,589 inbred lines to the susceptible inbred tester FR1064 and found significant genetic variation for both *Fusarium* ear rot and fumonisin concentration, but no complete resistance to either. This analysis demonstrated nearly complete dominance or overdominance of fumonisin concentration resistance alleles. Robertson *et al.* (2006) tested two segregating populations of $BC_1F_{1:2}$ and F_7-F_8 families, respectively, derived from the resistant lines GE440 and NC300. Heritability estimated on entry mean basis ranged from 0.75 to 0.86 for fumonisin concentration and from 0.47 to 0.80 for ear rot resistance. The higher heritability values obtained in the study of Robertson *et al.* (2006) compared to those obtained in Perez-Brito *et al.* (2001) may occure because the first authors used two artificial inoculations per plant and three to four evaluation environments, while in the second work one inoculation per plant and two testing

environments were employed. Moderate to high heritabilities suggest that ear rot and fumonisin contamination resistance should respond well to selection using family means.

The phenotypic correlation between *Fusarium* ear rot and fumonisin concentration has been reported to be moderate to low (Clements *et al.*, 2003; Clements *et al.*, 2004). In these studies, the correlations reported were affected by covariances between genetic, genotype x environment and experimental error effects on these two traits. Moreover, fumonisin concentration is generally greater in symptomatic kernels than in asymptomatic kernels (Desjardins *et al.*, 1998). Maize is often colonized endophytically by *Fusarium* spp. (Oren *et al.*, 2003), and it has been argued that significant amount of fumonisin can be produced in symptomless or only slightly rotten grain (Munkvold and Desjardins, 1997). Therefore, the correlations reported do not necessary provide an accurate indication of the magnitude of response of resistance to fumonisin concentration that would occur when selecting for ear rot resistance. The genotypic and phenotypic correlations between fumonisin concentration and ear rot were estimated by Robertson *et al.* (2006) in two populations of maize. Genotypic correlation was higher than phenotypic correlation (0.87-0.96 *vs.* 0.40-0.64) between ear rot and fumonisin concentration, indicating that genotypic effects on susceptibility to ear rot and fumonisin concentration are highly correlated, but genotype x environment and error effects are not highly correlated between the two traits. The close correlations between *Fusarium* ear rot caused by *F. verticillioides* and fumonisin contents suggest that toxin analysis is only exceptionally needed. This indicates that the genetically controlled mechanisms of resistance of these two aspects of disease are largely the same, and that selection for ear rot should affect fumonisin concentration and vice versa. Thus, from a breeder's point of view, selecting against ear rot may be a useful strategy for selecting genotypes with less genetic susceptibility to high fumonisin concentration.

1.3.5.1 Molecular markers and QTLs mapping

It's very hard to obtain high level of genetic resistance to *F. verticillioides* in maize. Evident progress have been reached with *Gibberella* ear rot and *Aspergillus* ear rot, but not in *Fusarium* ear rot. Many quantitative genetic variations for *Fusarium* ear rot resistance and fumonisins contamination resistance are present in different genotypes of maize and both disease traits have moderate to high heritability. As a consequence phenotypic selection should be an efficient way to improve resistance.

Unfortunately phenotypic selection for the two traits is made problematic by practical difficulties. Although many diseases could be evaluated during the plant young stage or before flowering, *Fusarium* ear rot and mycotoxins concentration can be analyzed only on mature seeds. In addition two liquid inoculations with calibrated fungine spores concentrations should be led to have a consistent evaluation of ear rot (Clements *et al.*, 2003). Therefore for the estimation of fumonisins contamination, are necessary time consuming and expensive toxin assays on precisely weighted and ground seed samples. Finally, many enviromental factors influence *Fusarium* ear rot and fumonisins contamination and locations that promote disease are required (Shelby *et al.*, 1994).

The availability of PCR-based DNA markers associated to resistance genes could be a successful strategy to select lines resistant to *F. verticillioides* (Robertson *et al.*, 2005). QTL mapping provides a powerful method to understand the genetic relationships between correlated traits, although they are hard to obtain for low heritability traits (Beavis, 1998). Pérez-Brito *et al.* (2001) showed that resistance to *Fusarium* ear rot is polygenic and QTLs were not consistent in the two populations analyzed. Robertson-Hoyt *et al.* (2006) reached different conclusions: they examined two segregating populations, GEFR and NCB, derived from the crosses GE440 x FR1064 and NC300 x B104. GE440 and NC300 were identified as sources of resistance. Three QTLs for ear rot and two QTLs for fumonisins contamination resistance were identified and they mapped in similar positions in the two populations. In particular, two QTLs were mapped on chromosome 4 and 5 and appeared to be consistent for both traits across populations. Therefore, two QTLs on chromosome 3 bin 3.04 were consistently identified across environments by Ding *et al.* (2008). The major resistant QTL with the largest effect explained 13-22% of phenotypic variation and it is flanked by SSR markers umc1025 and umc1742 at a distance of 3.4 centi Morgan (cM). The SSR markers closely flanking the major resistance QTL will facilitate marker-assisted selection (MAS) of resistance to *Fusarium* ear rot in maize breeding programs.

In order to determine the effectiveness of selection against ear rot for improving resistance to fumonisin contamination several selection procedures can be applied. Eller *et al.* (2008b) used advanced backcross lines containing alleles from GE440 (which has good resistance but poor agronomic quality) introgressed into an elite genetic background that lacks effective resistance (FR1064). The advanced backcross lines will represent a valuable resource for developing near-isogenic lines

(NILs) for finer-scale genetic analysis of the resistance QTLs previously mapped. An independent test of the hypothesis that selection against ear rot will improve fumonisin contamination resistance is being conducted by Eller *et al.* (2008a) via recurrent S_1 selection in a genetically broadbased population. The results of these two experiments should provide more definitive understanding of the genetic relationship between ear rot and fumonisin contamination resistances.

The pedigree method was also used for improving resistance to *Gibberella* ear rot in four maize populations (Presello *et al.*, 2005). Responses to selection were more evident in later than in earlier generations for both silk and kernel inoculation. Selection after kernel inoculation seemed to have been more effective than selection after silk inoculation in developing families with more stable resistance. It is concluded that responses to family selection could be accelerated without increasing operational costs by increasing selection intensity in later generations and inoculating fewer plants per family.

1.3.5.2 Transgenic approaches

Transgenic approaches to reduce *Fusarium* ear rot/mycotoxin resistance in maize are now availables. These comprise genetically resistance to insect feeding, increased fungal resistance and detoxification/prevention of mycotoxins in seed (Duvick, 2001).

Insects represent the main cause in fungal diffusion and following infection through kernels wounded by insect. In transgenic maize hybrids genes coding for insecticidal proteins from *Bacillus thuringiensis (Bt*) have been expressed (Hofte *et al*., 1989). The *Bt* gene encodes for the δ-endotoxins Cry1Ab and Cry1Ac, that confer protection against spike damage and reduce fumonisins levels. *Bt* maize has been widely sowed in the United States since 1997 and is a valid tool to reduce *Fusarium* ear rot and mycotoxins contamination.

The second strategy, that is transgene-enhanced disease resistance, has not obtained the same success than *Bt* maize. The reason is due to the reduced knowledge of the basic biology on the maize-*Fusarium* interactions. Genetic engineering could introduce proteins with antifungal activities to augment pathogen resistance, as reported for several leaf pathogens in dicotiledons (Munkvold and Desjardins, 1997). Unfortunately antifungal proteins are much less potent against their targets than Bt proteins and they should be expressed at high level to reduce fungal growth in tissue. In maize endosperm a cytosolic albumin called b-32, with a molecular

weight of 32 kDa, is synthesised in temporal and quantitative coordination with the deposition of storage proteins. This protein has homology with several previously characterized Ribosome-Inactivating Proteins (RIPs). Maize plants expressing a chimeric gene containing the *b-32* coding sequence downstream of a constitutive *35SCaMV*, have an increased tolerance to *Fusarium* attack in leaf tissue bioassays (Lanzanova *et al.*, 2009). The maize b-32 is an effective antifungal protein by reducing *Fusarium* infection progression.

Also enzymes involved in secondary metabolite synthesis in response to infection have been cloned (Frey *et al.*, 1997) and could be modified and overexpressed in maize. The limit in this strategy is the possibility that other biosynthetic pathways can be altered introducing new antifungal metabolites. Many experiments are focusing on the detection of expression changes in the numerous genes simultaneously, involved in defense pathways and on their control in maize (Baldwin *et al.*, 1999).

Although many defense pathways were identified in maize (Morris *et al.*, 1998), it is necessary to understand if these defense routes supply the real regulation against pathogen. Therefore these studies should be led in developing seed tissues, because researches about germinating seeds have indicated that defense gene expression can be improved by elicitor application following germination (Reddy *et al.*, 1999).

In addition an alternative strategy to confer resistance to *F. verticillioides* through protein engineering/directed evolution (Stemmer, 1994) is to identify new dominant resistance genes (*R* genes), that can help maize to recognize the pathogen in a gene-specific way through elements secreted by the fungus during plant infection.

A way to reduce mycotoxins contamination could be the use of strategies that suppress the synthesis of the mycotoxin itself or degrade it to nontoxic metabolites. For example transgenic plants could produce specific signals to turn off the mycotoxin pathway in the pathogen or to be defective in some essential signals for toxin production. Shim *et al.* (1999) have demonstrated that nitrogen repression controls fumonisins biosynthesis by *F.verticillioides*, although it is difficoult to manipulate this pathway without other effects on kernels. An approach to decrease mycotoxin accumulation is also the employment of catabolic enzymes to detoxify the mycotoxins *in situ* before their accumulation in plant. Many studies have focused their attention on the overexpression of plant genes coding for detoxification enzymes, that reduce disease. Few informations are available about fumonisins localization in maize, therefore recent experiments have showed that

fumonisins look resistant to the activity of known esterases and amine-modifying enzymes (Murphy *et al.*,1996). Many fungal species that metabolize fumonisins have been detected. For example *Exophiala spinifera* and *Rhinocladiella atrovirens* grow on FB_1 and produce enzymes that metabolyze the C-20 backbone portion of the fumonisin (Duvick *et al.*, 1994). In particular the enzymes that take part to the catabolic processes (deesterification and oxidative deamination) for detoxify FBs have been identified and the genes encoding for them have been cloned. The evaluations about their expression effects on FB levels in transgenic maize kernels are pursueing intensively.

Chapter 2

Aim of the Thesis

Legal limits for fumonisins presence in maize and its processed products are in force since 2007 (EU Regulations 1881/2006 and 1126/2007). These mycotoxins are responsible for several health problems in animals and humans. Therefore, it is urgent for maize producing countries to maintain fumonisins contamination below the fixed limits. Maize hybrids resistant to *F. verticillioides*, the fungus responsible for fumonisins production, are not available actually. Preventive actions are limited to the adoption of proper cropping systems, that are not sufficient to solve the problem. Although some studies about maize-*F. verticillioides* interactions were led, the molecular mechanisms regulating fungal activity and induced plant response are still unexplained. Their knowledge could contribute to fill these gaps and update management strategies.

Objective of this thesis is to apply transcriptomic studies to identify new host genes that contribute to the resistance against *F. verticillioides* infection.

In this study we used maize gene chips to analyse differential gene expression of maize kernels in response to *F. verticillioides*. We attempted to identify genes that may be involved in *Fusarium* ear rot resistance using resistant and susceptible maize genotypes.

In the first part of the study (**Experiment 1**), differentially expressed genes were identified at 48 hours after infection when fungal hyphae growing at the surface of the seed were clearly visible. The progression of the infection was investigated also on silks at various time points after infection.

Furthermore, in a second part of the work (**Experiment 2**), the microarray expression analysis was extended both to early and late phases of infection (from 12 to 96 hours after infection). With respect to a wild type *F. verticillioides*, a mutant strain unable to produce fumonisin was used in order to compare the host response.

Finally, a third experiment (**Experiment 3**) was performed in order to confirm the data obtained previously on a large number of resistant and susceptible maize genotypes infected by different mycotoxin producing fungal species, using the qRT-PCR analysis.

Chapter 3

Materials and Methods

3.1 Plant material

Two maize genotypes with contrasting phenotypes for resistance to *Fusarium* ear rot were used in the **Experiment 1, 2** and **3**: the resistant line CO441 and the susceptible line CO354. CO441 is a short-season corn inbred line derived from Jacques 7700 x CO298 with improved resistance to silk infection by *F. graminearum*. Resistance to infection via wounded kernels is also high (Reid *et al.*, 2003).

CO354 is susceptible to gibberella ear rot via silk channel inoculation and derived from Asgrow RX777 (Reid *et al.*, 2009). Both the lines were developed by the Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada (AAFC), Ottawa, Canada.

Therefore, in **Experiment 3,** the following inbreds were used, in addition to CO354 and CO441: CO387, CO388, CO389, CO430, CO431, CO432, CO433 and GT-MAS:gk. The CO lines were obtained from AAFC, Canada. The GS-MAS:gk population was provided in 1999 by USDA-ARS, Insect Biology and Population Management Research Laboratory, Tifton, GA. An inbred line was derived after several selfing generations and used in this experiment.

For microarray experiments, seeds of the lines were planted in pots (40 cm diameter, 35 cm height) and 10 plants of each line were grown up. Before infection, pots were transferred to an environmentally controlled greenhouse with day-time conditions of 28°C temperature and night-time conditions of 20°C, and a light regime of 16 h using lamps (Master TLD 58 W/830, Royal Philips Electronics, Eindhoven, The Netherlands) at intensity of 500 μmol m^{-2} s^{-1} (Figure 3.1).

Fig. 3.1 Maize genotypes grown up in greenhouse.

3.2 Inoculum production, infection and tissue sampling

F. verticillioides strain ITEM 1744, a prolific producer of fumonisins, was used for kernel inoculations of **Experiment 1**.

In **Experiment 2** we used the wild type *F. verticilliodes* strain ITEM 10514, a fumonisin-producing strain, and its corresponding mutant ITEM 10515 *FUM*1^{-}, a fumonisin-nonproducing strain, generated from ITEM 10514 by partial deletion of the *FUM*1 gene.

For **Experiment 3** the following strains were utilized: *F. verticillioides* ITEM 1744 producer of fumonisins, *F. proliferatum* ITEM 1719 producer of fumonisins, beauvericin and fusaproliferin, *F. subglutinans* ITEM 1521 producer of beauvericin and fusaproliferin and *Aspergillus flavus* ITEM 8096, producer of aflatoxins. All items were obtained from the Institute of Sciences of Food Production, National Research Council, Bari, Italy.

Working cultures were performed in collaboration with the Institute of Entomology and Plant Pathology in Piacenza. Cultures were maintained on Petri plates (9cm diameter) in Potato Dextrose Agar (PDA; infusion from potatoes 200 g; dextrose 15 g; agar 20 g; H_2O to 1L) for *F. verticillioides*, *F. proliferatum* and *F. subglutinans* and in Czapek Agar (CZ; sucrose 30 g; $NaNO_3$ 2 g; KCl 0.5 g; $MgSO_4.7H_2O$ 0.5 g; Fe $SO_4.7H_2O$ 0.01 g; K_2HPO_4 1 g; $ZnSO_4.7H_2O$ 0.001 g; $CuSO_4.7H_2O$ 0.005 g; agar 15 g; H_2O to 1L) for *A. flavus*, and incubated at 25 °C with a 12 h photoperiod for 14

days. Conidia were collected by rinsing plates with sterile water, scraping the agar surface with a scalpel and filtering the conidia suspension through sterile cloth. Spore suspension was obtained by adding 200 ml of sterile water to a final concentration of 10^6 conidia/ml based on microscopic counts using a Burcker chamber.

For the evaluation of inbred lines, 10 ears per plot were inoculated at 15 days after pollination (DAP) with a suspension of 2×10^6 conidia/ml of the toxigenic strain ITEM 1744 of *F. verticillioides*, using the side needle inoculations technique. Ear rot severity was evaluated based on a scale of 1–7, where 1 = no symptoms and 7 = more than 76% of the ear exhibiting symptoms.

The percentage of *F. verticillioides* was determined on 50 maize kernels for each sample according to Burges (1988). Fumonisins were analyzed by HPLC according to the method proposed by Shepard *et al.* (1990). Each sample consisted of seeds collected from each plot, dried at 40 °C. Sub-samples of 0.5 kg were ground for HPLC analysis.

Maize ears for microarray analysis were inoculated at 15 DAP using a pin-bar inoculator. The inoculating device consists of two 100 mm-long rows of 10 needles mounted on a wooden bar. Pins were dipped in conidial suspensions of 3.5×10^6 microconidia/ml and the bar was pressed through the husks sideways and into the centre of the ear, penetrating the kernels to a depth of 5–10mm (Figure 3.2).

In **Experiment 1** the inoculated ears were harvested 48 and 96 h after infection, and non-inoculated ears were also collected and served as controls. Kernels were sampled taking both seeds in the point of infection and seeds around the point of infection. For each time point, two biological replicates were taken in parallel.

For **Experiment 2** inoculated seeds were collected at 12, 24, 48, 72 and 96 h after infection. Kernels were sampled only in the area around the point of infection, to evaluate fungal development and diffusion. Three biological replicates were taken for each time point.

In **Experiment 3** infected seeds were harvested at 72 h after infection, as in **Experiment 1**. At least three biological replicates were collected.

Seeds were isolated using a scalpel, frozen in liquid nitrogen and stored at −80 °C until biological analyses were carried out.

In **Experiment 1**, the same conidia suspension was used to infect silks 15 days after silking (DAS). Silks were sprayed, covered with plastic bags to provide high humidity and collected at 12, 24, 48 and 72 h after infection As for the seeds, non-

inoculated silks were sampled and used as controls; for each time point, two biological replicates were taken in parallel. The resulting samples were immediately frozen in liquid nitrogen and stored at −80 ˚C.

Fig. 3.2 Ears and kernels upon *F. verticillioides* infection using pin-bar inoculator. Arrows indicate several positions where the pin enters into the cob.

3.3 Total RNA extraction

The collected samples were ground in liquid nitrogen with a pestle and mortar and total RNA was extracted from 2.5 g of seeds and silks respectively using Trizol protocol (Invitrogen, Carlsbad, CA, U.S.A.). RNA was then purified with the RNA Cleanup protocol (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. The amount and the quality of the total RNA were estimated by fluorimetric assay (Qubit, Invitrogen) as well as by agarose gel electrophoresis.

3.4 Microarray hybridization

Maize oligonucleotide arrays (Maize Oligonucleotide Array Project, version 1, University of Arizona, Tucson, AZ, USA) were used for **Experiment 1**. The array

consists of 46.000 oligonucleotide probes, representing >30.000 identifiable unique maize genes.

In **Experiment 2** we used the SAM 1.2 Chip, a 15,680 element cDNA microarray chip generated at Iowa State University's Center for Plant Genomics, USA.

Total RNA was reverse transcribed following the protocol of the Superscript Direct Labeling Kit (Invitrogen). After adding 5 μg of anchored Oligo(dT)20 Primer, 40 μg of total RNA was incubated at 70°C for 10 min and then placed on ice for at least 1min. The 20 μl final volume of reaction mix included 5X First-Strand buffer, 0.1M DDT, dNTP Mix for labeled dCTP, RNaseOUT^{TM}(40U), labeled dCTP (1mM) Cy3-Cy5 (GE Healthcare, Little Chalfont Buckinghamshire, UK), Super-Script^{TM}III RT (400 U). Incubation was performed in a water bath for 3 h at 46°C to generate high-quality labeled cDNA with high levels of fluorophor incorporation. RNA was degraded by alkaline treatment: 2 μl of 2.5M NaOH was added to the reaction mixture followed by incubation at 37°C for 15 min. The pH was immediately neutralized by adding 10 μl of 2M HEPES free acid to each reaction tube.

Unincorporated CyDye-nucleotides and short RNA oligomers were removed by column purification (Purification Module, Invitrogen). The cDNA labeled with Cy3 and Cy5 was lyophilized for 1.5 h and resuspended in 1X hybridization buffer (GE Healthcare) for a final volume of 20 μl.

The maize oligonucleotide arrays used for **Experiment 1** were pre-treated with a pre-hybridization buffer (at 1% SDS) for 5 min at room temperature, while SAM 1.2 Chips used for **Experiment 2** were pre-treated with a pre-hybridization buffer (at 5X SSC, 0.1% SDS and 0.01% BSA) for 45 min at 42°C.

The cDNA labeled targets were denatured at 95°C for 3 min and hybridized on a glass slide. A cover glass (Hybrislip, Sigma, St.Louis, MO, USA) was positioned on the spotted area and the slide was placed in a hybridization chamber (Corning Inc. Life Sciences, Acton, MA, USA). Hybridization was performed for 16 h at 56°C for the oligonucleotide arrays in **Experiment 1** and at 42°C for the SAM 1.2 Chips used in **Experiment 2**.

After hybridization, in **Experiment 1** slides were washed once at 55°C with 2X SSC buffer containing 0.5% SDS for 5 min, once at room temperature with 0.5X SSC for 5 min and once at room temperature with 0.05X SSC for 5 min. In **Experiment 2** slides were washed once at room temperature with 1X SSC buffer containing 0.2% SDS for 2 min, once at room temperature with 0.1X SSC buffer containing 0.2% SDS for 2 min and once at room temperature with 0.1X SSC for 2 min.

Finally, the slides of both experiments were placed in sterilized water before drying by centrifugation at 800 rpm for 3 min.

3.5 Microarray experimental designs

For the **Experiment 1** gene expression analysis was performed as follows:

Table 3.1 Comparisons of **Experiment 1**

CO441 non-inoculated vs CO441 48 h inoculated
CO354 non-inoculated vs CO354 48 h inoculated
CO441 non-inoculated vs CO354 non-inoculated
CO441 48 h inoculated vs CO354 48 h inoculated

Each experiment was carried out in two biological replicates and dye swaps (four hybridizations per comparison). Only RNA isolated from seeds 48 h after infection in both maize lines were used to perform microarray experiment, because RNA isolated from seeds 96 h after infection was degraded and not exploitable for **Experiment 1**.

In the **Experiment 2** the following comparisons were used for both the *F.verticillioides* strains, ITEM 10514 and 10515 (Table 3.2):

Table 3.2 Comparisons of **Experiment 2**

CO354 non-inoculated vs CO354 12 h inoculated
CO354 non-inoculated vs CO354 24 h inoculated
CO354 non-inoculated vs CO354 48 h inoculated
CO354 non-inoculated vs CO354 72 h inoculated
CO354 non-inoculated vs CO354 96 h inoculated

Each experiment was carried out in three biological replicates and dye swaps (six hybridizations per comparison).

3.6 Image acquisition, data collection and analysis

Using the GenePix 4000B AXON scanner and software (GenePix Pro 6.0, Axon Instruments, Union City, CA, USA) hybridized slides were scanned with two wavelengths corresponding to the dyes used. Scanning was carried out at 5 μm resolution and PMT was adjusted for both channels, to obtain the ratio of medians of control spots near 1. The data were normalized for each array separately (print-tip loess and global loess for the first and second experiments, respectively) to compensate for differences in sample labeling and other non-biological sources of variability. Raw data were then processed in Limma, taking advantage of the interface LimmaGUI. The Linear Model described in Limma was used to identify probes showing significant differential gene expression in the array comparisons (Smyth *et al.*, 2005). To identify statistically significant differentially expressed genes, p-value lower than 0.01 was used as criteria in Experiment 1 and 2. In the Experiment 1, the induction or repression ratio higher than 2-fold change (FC) was used as additional criteria (log FC $\geq$ 1.4).

3.7 qRT-PCR expression analysis

Gene expression data from microarray hybridizations were validated in quantitative reverse transcriptase (qRT)-PCR analysis. This was performed on the most interesting genes up- or down-regulated during the infection in CO441 and CO354 lines evidenced after microarray hybridization. Some of these genes were also tested in silks of both maize lines in **Experiment 1**. In **Experiment 3,** the gene *PRm*6 was tested in the kernels of the lines CO354, CO387, CO388, CO389, CO430, CO431, CO432, CO433, CO441 and GS-MAS:gk infected by *F. verticillioides*. The same gene was tested in CO354 and CO441 kernels infected by *F. proliferatum*, *F. subglutinans* and *Aspergillus flavus*.

A 1-μg sample of total RNA was used for cDNA synthesis following the iScript cDNA synthesis kit protocol (Bio-Rad, Hercules, CA, USA). 20 ng of single strand cDNA determined by fluorimetric assay (Qubit, Invitrogen) were used for qRT-PCR. The reaction mix contained 2X iQ SYBR Green Supermix (Bio-Rad) and 0.4 μM of each primer added to cDNA. Relative quantitative analysis was performed using the MiniOpticon device (Bio-Rad) under the following conditions: 95°C for 3 min and 44 cycles at 95°C 10 s, 60°C 25 s. A melting curve analysis, ranging from 60 to 95°C, was used to identify different amplicons, including non-specific products. Three replicates were employed for each tested sample and template-free

negative controls. The *β-actin* and the *Elongation factor* (EF)-1α were used as internal control for seeds and silks respectively, to normalize all data. Gene-specific primers were designed within consecutive exons, separated by an intron, using Primer3 software and their sequences are shown in Table 3.3. Relative quantification was normalized to the housekeeping control genes and the expression ratio and FC were calculated using the $2^{-\Delta\Delta Ct}$ method.

In **Experiment 1** to quantify the growth of *F. verticillioides* the copy number of *β-tubulin* 2 and *FUM* 21 genes was detected using qRT-PCR. The primer pairs BT F1 and BT R2 were designed within a conserved region positioned between nucleotides 12 and 75 of the *β-tubulin* 2 sequence (NCBI GenBank Accession No. GQ915447.1). The primers FUM21 F3 and FUM21 R3 were used for amplification of the *FUM* 21 gene sequence (NCBI GenBank Accession No AF155773.5). The PCR thermal cycling conditions were as indicated above. The copy number of *β-tubulin* 2 and *FUM* 21 was determined based on the equation of the linear regression according to the technical manual (Bio-Rad).

In **Experiment 2** the copy number of the *β-tubulin* 2 was evaluated in the lines CO354 and CO441 infected by the wild type and mutant strains of *F. verticillioides*.

Table 3.3 Primers used for qRT-PCR analysis in **Experiment 1**, **2** and **3**.

Annotation	**Primer Forward**	**Primer Reverse**
Pathogenesis-related protein 1	GAACTCGCCGCAGGACTAC	GAGCCCCAGAAGAGGTTCTC
Pathogenesis-related protein 5	GTCATCGACGGCTACAACCT	GGGCAGAAGGTGACTTGGTA
1,3-β-glucanase PRm6	GCGCAGACCTACAACCAGA	GGAGAAATTGATGGGGTACG
Thaumatin-like protein	CCGTGTTCAAGAAGGACGAG	GGGCAGAAGACGACCTTGTA
Chitinase PRm 3	GGCTCTACGCCTACGTCAAC	GATGGAGAGGAGCACCTTGA
β -glucosidase, root meristem	ACATCACGGAGAACGGAATC	AGCGAAGTAGCCTTGCACAT
Respiratory burst oxidase protein B	TGCTTGCATTATGGATTGGA	TTACGGCATATTGGGAGGAG
Bowman-Birk proteinase inhibitor WIP1	ACACCTGGTGCTGATCCTGT	CCGAGAAGGAGAAGTTGCAG
Putative peroxidase	GCACAAGGTCCTGTTCGTCT	TTTCCCTGATCTCTCCCTCA
Glyceraldehyde-3-phosphate dehydrogenase	CTCCGCTTGCTAAGGTCATC	TGCTGGGAATGATGTTGAAA
β-actin	ATGGTCAAGGCCGGTTTCG	TCAGGATGCCTCTCTTGGCC
Elongation factor 1 α	GCTTCACGTCCCAGGTCATC	TAGGCTTGGTGGGTATCATC
β-tubulin	AGACCGGTCAGTGCGGTAAC	CGTGCTCGCCAGAGAGATGGT
*FUM*21	ATGCAGATCCGGGAAGGTGTTC	TGTAATCTCGTCTGCAATCAAATCC
WRKY1	GAGGAGCAATCTTGGGATCA	CAAGACTCCCTTCCATGCTC
Myb-like DNA binding protein	ATACCTGCCCTGGAGAACTG	AGTCGATCATGCTGGTGGAT
PDR6 ABC-transporter	TGGCGTCAAGTACAGAATCG	AGCATGCTTTTCAAGGGAAG
β -expansin 1 protein	GCCTCCGTTTATTTGCAGAC	TAATAGCGCGGGTTTTATCG
Hypersensitive-induced response protein	CAGACCAGCCTTACCACCAC	GCATTTGCTCCTGGGAATTA
Calcineurin B protein	AATTGCGCCATTCTTCTTTG	TGCTACACTTGCGGAGTCAG

Chapter 4

Results and Discussion

4.1 Experiment 1

To gain an understanding of the relationships between fungal infection and transcripts abundance in maize during *F. verticillioides* infection, a microarray analysis was performed. The host defense response induced during the infection was investigated in susceptible and resistant maize genotypes. The maize-*F. verticillioides* interaction was analyzed at an intermediate stage from 0 to 48 h after infection, in the point where the infection took place.

4.1.1 Genotype response to *Fusarium verticillioides* infection

Significant differences between inbreds were detected for ear rot severity, incidence of kernels infected by *F.verticillioides* and total fumonisin content. Inbred CO441 displayed 1.7 ± 0.8 % infected kernels compared to 82.9 ± 7.8 % infection for the suscettible inbred CO354 over the two years. The mean *F. verticillioides* kernel infection was 16.0 ± 7.0 % and 80.0 ± 20.0 % for the resistant and susceptible inbreds respectively. The resistant inbred had a low level of total fumonisins (1.63 ± 0.7 mg/kg) compared to the susceptible one (7.45 ± 1.3 mg/kg). Pin-bar inoculation proved to be a useful technique for identifying resistant genotypes allowing high *F.verticillioides* growth.

4.1.2 Detection of *Fusarium verticillioides* in seeds and silks

F. verticillioides growth was assayed by absolute quantification of the *tub* 2 gene through qRT-PCR. The results of the qRT-PCR analysis are shown in Figure 4.1A. We observed that, in the infected kernels of the resistant maize line 48 h after infection, the copy number of *tub* 2 was about three times lower (602.0 copy number ± 18.1 *vs.* 1861.0 copy number ± 87.7) than that found in the susceptible kernels. At 96 h after infection total RNA was degraded and the subsequent qRT-PCR was not performed.

In silks, *tub*2 was not detectable at early stages (from 12 to 48 h) of infection. *Tub*2 was first detected at 72 h after infection, although in a reduced amount (7.0 copy number ± 2.1 in average) and differences between lines were not significant.

The *FUM*21 gene encodes a putative Zn(II)2Cys6 DNA-binding transcriptional activator that is likely to be specific for *FUM* cluster genes. A functional *FUM*21 is

necessary for fumonisins production in *F. verticillioides* (Brown *et al.*, 2007). The results obtained for the *FUM*21 gene were similar to those obtained for *tub* 2, although the relative copy number was lower than that observed for *tub*2 (Fig. 4.1B). In infected seeds of the susceptible line, the *FUM*21 copy number reached a higher level compared to the resistant line (115.6 copy number ± 17.2 *vs.* 22.4 copy number ± 11.6). Resistant and susceptible silks showed no difference in *FUM*21 gene copy number (54.8 ± 35.6 *vs.* 41.3 ± 28.7, respectively).

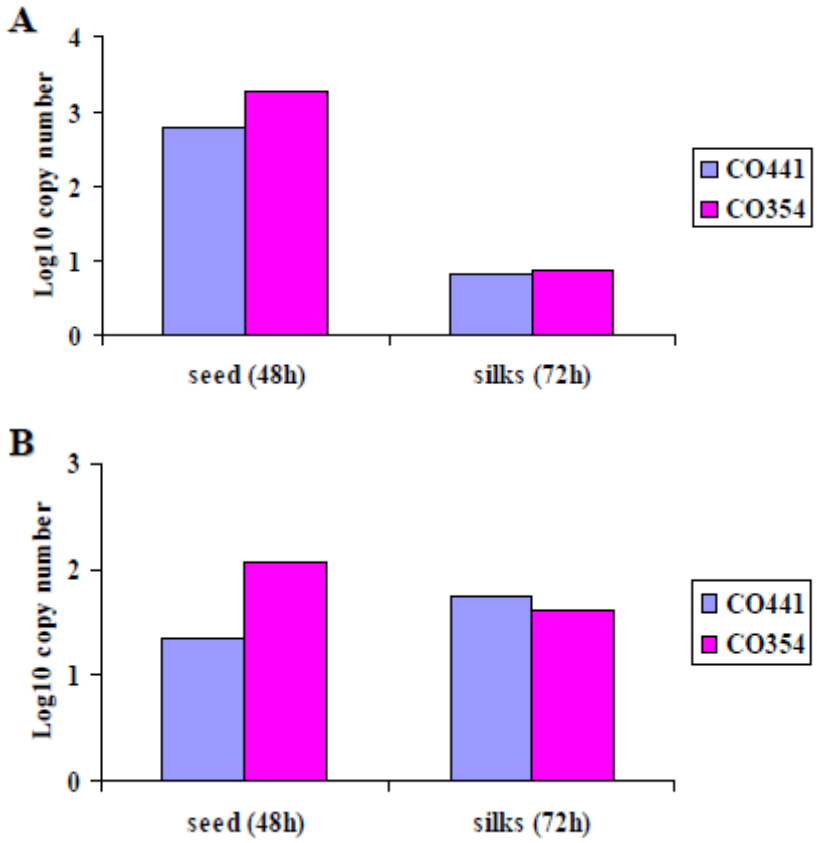


Fig. 4.1 A and **B** Logarithm of the copy number of *Fusarium verticillioides* genes in kernels and silks of the maize lines CO441 and CO354. (**A**) Copy number of the constitutive gene for ß-*tubulin* 2 and (**B**) of the transcriptional regulator *FUM*21.

4.1.3 Gene expression analysis in seeds infected with *Fusarium verticillioides* within maize genotypes

Gene expression data were obtained from microarrays hybridizations using total RNA extracted from maize seeds at 15 DAP. We used Maize Oligonucleotide Array version 1 to measure and compare the accumulation transcripts of more than 30.000 maize genes.

In the comparison between the control and 48 h after infection, 820 differentially expressed sequences with $p<0.01$ were identified in seeds of CO441; in the CO354

comparison, 673 differentially expressed sequences with $p<0.01$ were therefore highlighted (Supplementary Tables 4.1 and 4.2).

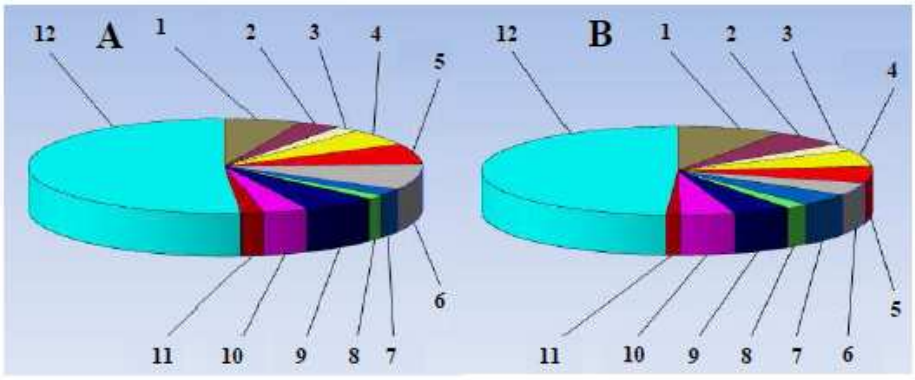
The differentially expressed sequences were classified according to the Munich Information Center for Protein Sequences (MIPS) FunCat database and sorted into 11 functional categories: Metabolism; Energy; Development; Cellular transport; Cellular communication; Cell rescue, Defense and virulence; Protein synthesis; Storage protein; Cell cycle and DNA processing; Transcription; Unclassified proteins (Unknown). Some cellular functions had a lower numeric relevance in our samples; these were grouped into a separate 12th functional class, named "Others", which included the following functional categories: "Protein fate, Protein with binding function, Regulation of metabolism, Transposable elements and Cell fate" (Figures 4.2 and 4.3).

Of the differentially expressed genes, a larger portion was unclassified with respect to the putative function. Of the genes up- and down-regulated in seeds of the line CO441 at 48h post-infection, 51.4% and 49.0% were unknown, respectively (Figure 4.2). Similarly, 48.1% and 49.3% respectively of the up- and down-regulated genes (Figure 4.3) were unknown in the susceptible genotype, suggesting that our understanding of the cellular processes occurring during infection is very limited. Functional categories that were enriched in up- and down-regulated genes in seeds may give an indication of some of the important molecular processes taking place during *F. verticillioides* infection. For example, the most representative functional class was "Cell rescue, defense and virulence", which included 9% of over-expressed genes in both maize lines. Comparing the percentages of up- and down-categories, the Cell cycle and DNA processing (6.2%) prevailed as up-regulated in CO441, while Metabolism, Energy and Protein Synthesis included most of the down-regulated genes (8.2, 6.0 and 4.5%, respectively). The genes showing higher expression in CO354 were those for Metabolism (6.0%) and Storage Protein (4.1%). Functional categories that were enriched in CO354 down-regulated genes were Development (4.6%), Protein Synthesis (5.9%), Cell Cycle and DNA processing (6.2%) and Transcription (4.6%).

All the differentially expressed genes are reported in Supplementary Material with the corresponding p- values.

In the following sections we discuss aspects of the seed-specific expression profiles for maize genes encoding proteins related to cell defense, metabolism and

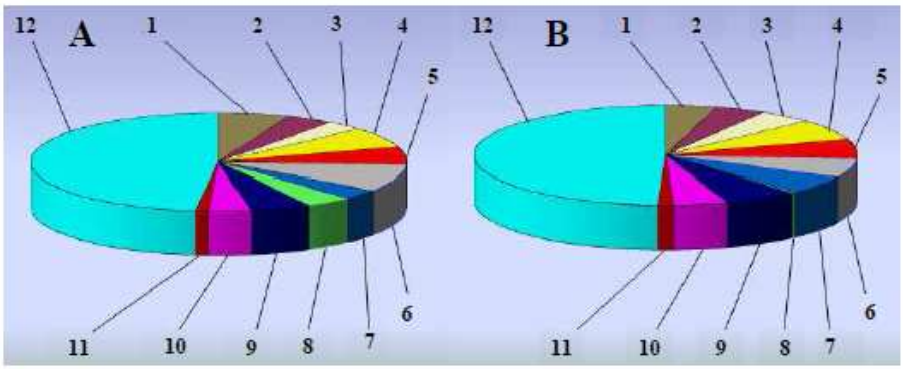
development. We selected differentially expressed genes involved in response to fungal infection, comparing controls and infected kernels in both maize lines.



C

Functional category	Up-regulated	Down-regulated
(1) Metabolism	6.2%	8.2%
(2) Energy	3.5%	6.0%
(3) Development	2.0%	2.6%
(4) Cellular transport	5.7%	5.9%
(5) Cellular communication	6.9%	6.2%
(6) Cell rescue, defense and virulence	9.0%	4.5%
(7) Protein synthesis	2.5%	4.5%
(8) Storage protein	1.5%	1.9%
(9) Cell cycle and DNA processing	6.2%	4.8%
(10) Transcription	3.7%	4.5%
(11) Others	1.7%	1.2%
(12) Unknown	51.4%	49.0%

Fig. 4.2 Genes with significantly (A) higher and (B) lower expression in kernels of CO441 at 48 h after *Fusarium verticillioides* infection. Genes are grouped according to their predicted function. (C) Percentage of genes with either higher or lower expression, for each functional group.



C

Functional category	Up-regulated	Down-regulated
(1) Metabolism	6.0%	4.2%
(2) Energy	4.1%	4.2%
(3) Development	3.3%	4.6%
(4) Cellular transport	6.8%	6.9%
(5) Cellular communication	5.7%	6.2%
(6) Cell rescue, defense and virulence	9.0%	6.2%
(7) Protein synthesis	3.3%	5.9%
(8) Storage protein	4.1%	0.3%
(9) Cell cycle and DNA processing	5.2%	6.2%
(10) Transcription	3.5%	4.6%
(11) Others	1.1%	1.3%
(12) Unknown	48.1%	49.3%

Fig. 4.3 Genes with significantly (A) higher and (B) lower expression in kernels of CO354 at 48 h after *Fusarium verticillioides* infection. Genes are grouped according to their predicted function. (C) Percentage of genes with either higher or lower expression, for each functional group.

4.1.3.1 Cell defense

Of the twelve differentially regulated categories, genes assigned to the category Cell rescue, defense and virulence were predominantly represented by up-regulated genes in both resistant and susceptible maize lines. These genes encode to a large extent for PR proteins (Table 4.1). The most significant over-expressed PR genes were: the chitinase or PRm3 (MZ00034300), which reduces the content of chitin in the fungal membrane (Van Loon and Van Strier, 1999); the PR-related protein (MZ00036176), identified for the first time in *Oryza sativa*; the PR 4 (MZ00043659), first identified in *Triticum monococcum*; the PR 5 (MZ00035052), also named permatin for its ability to determine fungal hyphae leak and rupture (Morris *et al.*, 1998); the thaumatin-like protein (MZ00036117), which is expressed in response to a variety of infections, stress and developmental signals. Several genes related to defense were involved in the detoxification response: the putative cytochrome P450 (MZ00056561), the cytochrome P450 monoxygenase (MZ00016340), the putative peroxidase (MZ00028399), the glutathione-S-transferase (MZ00037479), the respiratory burst oxidase protein B (MZ00003713) and the germin 4 (MZ00014560) with superoxide dismutase activity in response to extracellular superoxide radicals (Woo *et al.*, 2000). Other genes upregulated in response to fungal infection in both lines were identified as coding for members of the small and large heat-shock proteins (HSPs): the classes II 17.8 kDa (MZ00024484) and 82 kDa (MZ00023554), respectively. The largest may act as molecular chaperone by regulating folding of resistance (R) proteins (Shen *et al.*, 2007), while Z. *mays* HSP 17.8 is known to be heat-induced but not induced by other forms of stress (Waters, 1995). A Bowman-Birk type WIP1 proteinase inhibitor (MZ00014363) was simultaneously induced in both maize genotypes. This gene is known to be strongly induced by wounding and its product can be transmitted to the adjacent regions of the wounded area (Rhormeier and Lehle, 1993).

4.1.3.2 Cell metabolism

Examination of categories Metabolism, Energy and Protein synthesis, revealed a prevalence of down-regulation (Table 4.1, Fig. 4.2, Supplementary Table 1). Among the down-regulated genes of the three cited categories were some for enzymes involved in sugar metabolism –e.g. those encoding starch synthase (MZ00025889), aldolase (MZ00042886), glyceraldehyde-3-phosphate dehydrogenase (MZ00035263) and phosphoribosyl-pyrophosphate synthase (MZ00044555). This

observation suggests that gluconeogenesis and glycolisis are both repressed in seeds after fungal infection, although the aldolase activity has been reported as up-regulated in germinating embryos (Campo *et al.*, 2004). On the other hand, the cytoplasmic malate dehydrogenase enzyme (MZ00013626) was up-regulated in the susceptible line, indicating an increase in reducing equivalents between the cytoplasm and mitochondria (Yang *et al.*, 1993). Genes involved in protein biosynthesis, expression of which decreased significantly in infected seeds compared with control seeds, were identified as ribosomal (Supplementary Material). Three up-regulated transcripts encoded ß-glucosidases: the ß-glucosidase (MZ00039184), the glucan endo-1,3-ß-D-glucosidase (MZ00028849), and the ß-glucosidase aggregating factor (MZ00018944). The accumulation of multiple ß-glucosidases in the fungal-infected embryo tissues of barley was found (Nielsen *et al.*, 2006). The role of up-regulated ß-glucosidase genes during defense activation is still unclear, although ß-glucosidases fulfil important functions in the degradation of various biopolymers.

4.1.3.3 Cell development

Analysis of the genes assigned to the functional classes Development, Cell cycle and DNA processing, Transcription and Cell communication (Table 4.1, Fig. 4.2, Supplementary Table 1) showed that two transcriptional factors were down-regulated in the susceptible line CO354. One of these encodes for an EREBP (ethylene-responsive element binding proteins, MZ00024413), which is a prototypic member of a family of transcription factors unique to plants, forming an element of the mechanisms used by plants to respond to various types of biotic and environmental stress (Riechman and Meyerowitz, 1998). The second encodes the RING finger protein CNOT 4 (MZ00037026), which is a component of the CCR4-NOT complex and functions as ubiquitin protein ligase (E3). This complex is implicated in the repression of RNA polymerase II transcription (Albert *et al.*, 2002). This correlates with down-regulation of an RNA polymerase beta subunit (MZ00040526). Within this class we identified up-regulated genes that would fit into different transcriptional responses to the pathogen: a CAF protein (MZ00032902), a Dicer homolog, that acts in micro-RNA metabolism (Park *et al.*, 2002); a cnd41 (MZ00048814), a negative regulator of chloroplast gene expression (Nakano *et al.*, 1997); and a γ-tubulin 1 (MZ00044486), which participates in microtubules organization (Drykova *et al.*, 2003). The specific response to *F.*

verticillioides included repression of a growth regulating factor 1 (MZ00027809), playing a role in gibberellin-induced growth (van der Knaap *et al.*, 2000), and of senescence-associated protein 12 (MZ00042047), involved in the maintenance of cellular viability under nutrient-limited conditions (Doelling *et al.*, 2002). We found that few genes for putative calcium dependent protein kinases (MZ00023120 and MZ00001537) were also up-regulated. It has been reported that the activation of kinases is accompanied by an increase in the level of PR mRNA. A remarkable cell-type specific pattern of expression of the calcium-dependent protein kinase 10, in which the PR genes were also expressed, was described in response to *F. verticillioides* infection in maize seeds by Murillo *et al.* (2001).

Table 4.1 Selected maize genes differentially regulated after *Fusarium verticillioides* infection.

[a]Probe code. [b]Organisms other than *Zea mays* are reported in brackets.[c]Logarithm$_2$ Fold Change.

ID[a]	Annotation[b]	LogFC[C]	Functional
MZ00024484	17.8 kDa class II heat shock protein	3.72	Cell rescue, defense and virulence
MZ00014363	Bowman-Birk proteinase inhibitor WIP1	1.96	
MZ00034300	Chitinase PRm 3	2.01	
MZ00016340	Cytochrome P450 monooxygenase (*O. sativa*)	1.46	
MZ00037479	Glutathione S-transferase 41	3.24	
MZ00023554	Heat shock protein 82 (*O. sativa*)	1.47	
MZ00036176	Pathogenesis-related protein-like protein (*O. sativa*)	1.39	
MZ00043659	Pathogenesis-related protein 4 (*Triticum monococcum*)	2.41	
MZ00035052	Pathogenesis-related protein 5	1.85	
MZ00014560	Probable germin protein 4 (*O. sativa*)	1.49	
MZ00056561	Putative cytochrome P450 (*O. sativa*)	2.01	
MZ00028399	Putative peroxidase (*O. sativa*)	2.01	
MZ00003713	Respiratory burst oxidase protein B (*O. sativa*)	2.02	
MZ00036117	Thaumatin-like protein	2.53	
MZ00013626	Malate dehydrogenase, cytoplasmic	4.43	Metabolism
MZ00044555	Putative phosphoribosyl pyrophosphate synthase	-1.97	
MZ00025889	Starch synthase DULL1	-2.86	
MZ00039184	β-glucosidase, root meristem	1.65	
MZ00028849	Glucan endo-1,3- β-D-glucosidase (*Nicotiana tabacum*)	1.84	
MZ00018944	Putative β-glucosidase aggregating factor (*O. sativa*)	1.34	
MZ00032902	CAF protein (*Arabidopsis thaliana*)	2.24	Cell cycle and DNA processing
MZ00040526	DNA-directed RNA polymerase β-subunit (*Cusenta reflexa*)	-2.02	
MZ00044486	γ-tubulin 1	1.79	
MZ00048814	Putative nucleoid DNA-binding protein cnd41 (*O. sativa*)	2.66	
MZ00023120	Putative calcium-dependent protei kinase (*Oryza sativa*)	1.51	Cell communication
MZ00001537	Putative calcium-dependent protei kinase (*O. sativa*)	1.31	
MZ00027809	Growth-regulating factor 1 (*O. sativa*)	-1.48	Development
MZ00042047	Putative senescence-associated protein 12 (*A. thaliana*)	-1.90	
MZ00042886	Cytoplasmic aldolase	-2.06	Energy
MZ00035263	Glyceraldehyde-3-phosphate dehydrogenase	-1.77	
MZ00024413	EREBP-like protein (*O. sativa*)	-2.18	Transcription
MZ00037026	Putative CCR4-NOT transcription complex subunit 7 (*O. sativa*)	-3.36	

4.1.4 Gene expression analysis in seeds before and after infection with *Fusarium verticillioides* between maize genotypes

Gene expression patterns were surveyed among parallel control and infected ears of CO441 and CO354 at 48 h after inoculation. We designated a transcript as present when it showed a significant expression value in four replicates (p value $\leq E^{-02}$ and log FC ≥ 1.4). We identified a total of 739 probe sets that detected transcripts that were expressed differentially between the two inbred lines at one time point after infection (Table 4.2).

We conducted a broadbased classification of all the differentially expressed maize genes into 11 categories according to biological or molecular function. Annotation revealed that a wide variety of functional and structural categories were involved in response to *F. verticillioides* infection. Among all the differentially regulated genes, 7.3% of them encoded proteins that play a role in cell rescue and defense. About 16% of the differentially regulated genes were tallied in the cellular communication, cell cycle and DNA processing and transcription categories.

Transcripts analysis before infection

A higher number of over-expressed genes was called out in the line CO441 versus CO354 before infection, namely 330 genes (60% of the total number of tested sequences; Table 4.2). Within the up-regulated list, cell rescue and defense was the over-represented category with 33 transcripts (10% of total).

Closer observation of the transcripts accumulation group suggested that major changes in defense gene expression occurred between CO441 and CO354 lines before infection (Table 4.3). Among this category there were a number of genes encoding putative components of disease resistance such as chitinase, thaumatin, protease inhibitor, beta-1,3-glucanase and heat shock hsp22. The defense response class of genes included also proteins involved in the oxidative burst and in the detoxification of ROIs, such as genes for peroxidase, glutathione S-transferase, cytochrome P450, thioredoxin and cytochrome c reductase. Peroxidases would act both as basal defense components as well as activators of oxidases, such as NADPH oxidase, which are involved in ROIs production (Berrocal-Lobo and Molina, 2008). ROIs produced in the oxidative burst could serve not only as protectant against invading pathogen, but could also be the signals activating further plant defense reactions (Wojtaszok, 1997). Four genes, glutathione S-transferase (MZ00026782), ubiquinol-cytochrome c reductase (MZ00015131), antifungal thaumatin-like protein (MZ00000977) and subtilisin/chymotrypsin inhibitor (MZ00005959), exhibited the

highest value of transcripts accumulation. Few genes whose products were involved in signaling and regulatory components were up-regulated in CO441.

Transcripts analysis after infection

Examination of transcripts accumulation data of cell rescue and defense genes underlined a decreased number of up-regulated genes and a substantial increase in down-regulated transcripts (Table 4.2). Interestingly, the most relevant down-regulated genes included ten members of the category defense related proteins and seven genes involved in oxidative-burst and detoxyfication (Table 4.4). Genes that exhibited greater repression after infection were: two chitinases (mean log FC -2.22), a licheninase (-6.35) and a glucanase (-3.60). Seven genes of the category oxidative burst and detoxyfication (three peroxidases, three cytochrome-related proteins and a germin protein with SOD activity showed, in average, -1.76 fold change.

One probe encoding for 3',5'-flavonoid hydroxylase was also detected under-regulated. The 3',5'-flavonoid hydroxylase specific for maize kernels belongs to the CYP75 family of p450 proteins and performs hydroxylation of flavones and flavanones (Kaltenbach *et al.,* 1999).

Table 4.2. Number of up- and down-regulated genes among 12 functional categories, as determined by comparing CO441 vs. CO354 lines before (0 h) and after (48 h) infection.

	0 h		**48 h**	
Functional category	Up	Down	Up	Down
Cell rescue and defense	33	11	27	27
Cell cycle and DNA processing	21	10	30	20
Cellular comunication	20	12	18	25
Transcription	10	4	13	10
Metabolism	19	9	20	27
Energy	15	9	10	12
Cellular transport	15	13	20	23
Protein synthesis	12	7	11	24
Development	11	9	5	13
Storage protein	10	9	21	12
Others	5	4	11	12
Unknown	159	122	175	173
Total number of genes	330	219	361	378

Table 4.3. Defense response genes up-regulated determined by comparing not inoculated CO441 vs. CO354 lines.

ID	Annotation	logFC	p-value
	Genes for signalling and regulatory components		
MZ00021816	Zinc-finger protein	1.77	5.69E-03
MZ00037227	Zinc finger transcription factor ZF1	2.30	1.19E-03
	Defense related proteins		
MZ00000977	Antifungal thaumatin-like protein	2.86	3.52E-04
MZ00039524	BETL2 (BAP) protein	2.01	2.77E-03
MZ00004170	Chitinase III	2.27	5.84E-03
MZ00017997	Elicitor inducible beta-1,3-glucanase	1.56	9.20E-03
MZ00029223	Heat shock protein hsp22 precursor	1.61	7.62E-03
MZ00005959	Subtilisin/chymotrypsin inhibitor	4.38	8.13E-05
	Oxidative burst and detoxification		
MZ00017047	Cytochrome b5	1.57	7.23E-03
MZ00004887	Cytochrome P450	1.69	5.02E-03
MZ00020279	Cytochrome P450	1.75	5.46E-03
MZ00028884	Cytochrome P450 monooxygenase	2.37	9.34E-03
MZ00017025	Ferredoxin VI, chloroplast precursor	2.41	2.92E-03
MZ00026782	Glutathione S-transferase	3.48	1.81E-03
MZ00029219	Peroxidase	2.12	4.87E-03
MZ00033489	Peroxidase	1.81	3.97E-03
MZ00039972	Thioredoxin-like	1.88	3.48E-03
MZ00015131	Ubiquinol-cytochrome c reductase	3.37	2.67E-04

Table 4.4 Defense response genes down-regulated by comparing CO441 vs. CO354 lines at 48 h after infection.

ID	Annotation	logFC	p-value
	Genes for signaling and regulatory components		
MZ00035970	RING/C3HC4/PHD zinc finger protein	-1.40	8.47E-03
MZ00051123	RING zinc finger protein	-1.37	6.69E-03
MZ00012652	Zinc finger protein	-2.56	3.65E-03
MZ00037667	Zinc finger protein	-2.27	5.62E-03
	Defense related proteins		
MZ00043886	Acidic class I chitinase	-2.08	5.92E-04
MZ00041327	Bowman-Birk trypsin inhibitor	-1.72	7.66E-03
MZ00041276	Chitinase	-2.36	4.59E-03
MZ00004734	Disease resistance protein	-1.45	5.41E-03
MZ00001846	Disease resistance protein	-1.94	1.50E-03
MZ00039183	ß-D-glucosidase	-1.95	7.48E-03
MZ00056690	1,3-ß-glucanase PRm 6b	-1.57	4.49E-03
MZ00001210	Heat shock protein	-1.99	9.10E-03
MZ00037281	Licheninase precursor	-6.35	5.65E-06
MZ00031089	Xyloglucan endo-1,4-ß-D-glucanase	-3.60	9.34E-03
	Oxidative burst and detoxification		
MZ00029247	Cytochrome c	-1.77	2.40E-03
MZ00029836	Cytochrome P450	-1.84	1.61E-03
MZ00017465	Cytochrome P450-related protein	-3.00	6.02E-03
MZ00023152	Peroxidase precursor	-1.32	9.23E-03
MZ00044327	Peroxidase	-1.60	2.99E-03
MZ00052181	Peroxidase	-1.85	6.66E-03
MZ00041591	Germin protein 4	-1.48	8.50E-03
	Enzymes for secondary metabolism		
MZ00019364	Flavonoid 3',5'-hydroxylase	-1.50	7.17E-03

4.1.5 qRT-PCR of the defense-related genes in kernels

To validate the microarray results, eight differentially-expressed genes were selected for confirmation by qRT-PCR (Figure 4.4). The qRT-PCR results showed that the expression trends of these genes were almost consistent with the results from microarray analysis. However, given the greater sensitivity of qRT-PCR, the variation in differentially-expressed genes from qRT-PCR was greater than that from the microarray analysis. In this work we included a pair of PR genes (PR1 and PRm6) not detected as significantly expressed in the microarray experiment. Pathogen-induced accumulation of the nine transcripts reached higher levels in the susceptible genotype than in the resistant genotype at 48 h after infection (Figure 4.4A). Most of the pathogenesis-related genes were differentially activated after *F. verticillioides* infection depending on the resistance level of the maize genotypes (Figure 4.4B). PR1, PR5, PRm6, thaumatin, chitinase, burst oxidative protein, WIP1 and peroxidase were strongly up-regulated before *Fusarium* inoculation in the resistant genotype. Their synthesis is massively activated as a response to pathogen infection in the susceptible genotype. On the contrary, ß-glucosidase presented a high expression ratio before fungal attack in CO354. Altered gene expression in response to *Fusarium* spp. inoculation, which differed between genotypes, was found for wheat-*Fusarium* interaction (Steiner *et al*., 2008). The differential expression between wheat lines was associated with the possession of resistance genes and the *Fusarium* resistance level of the genotypes. The cytosolic glyceraldeide 3-phosphate dehydrogenase (G3PDH) emerged among the well-characterised genes known to be down-regulated in response to fungal infection (Campo *et al*., 2004). Indeed, the level of accumulation of this enzyme decreased in both maize genotypes after 48 h of infection, and the expression ratio in CO354 was less pronounced compared with the values of the other validated genes.

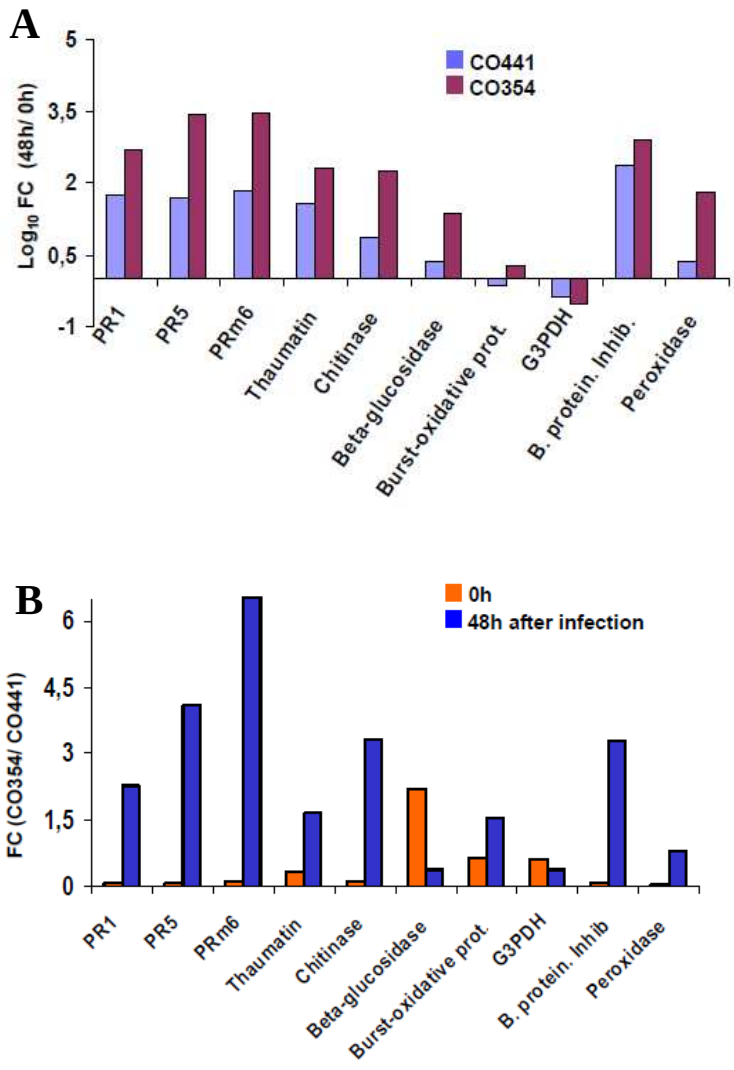


Fig. 4.4 A and **B**. Logarithm of fold change (FC) of differentially expressed genes in kernels of CO441 and CO354 at 48 h after infection with Fusarium verticillioides, compared to 0 h (A). FC of genes expressed before (0 h) and after (48 h) kernels infection in CO354 compared to CO441 (B).

4.1.6 Expression changes in defense genes in silks

In order to further explore the fungal-induced expression of defense genes in silks, qRT-PCR experiments using gene-specific primers were carried out. It is known that the most important pathway for infection is through silks, causing infection of 84% of kernels (Munkvold, 2003). Germinated spores enter kernels via silks. Silks become progressively colonized by the pathogen from the ear top to the base, but the entire length of the majority of the silks of an ear are infected only after 28 days (Headrick *et al.*, 1990). In agreement with these observations the presence of the fungus could not be detected before 72 h after infection, as reported above. In this

phase of infection we measured the expression ratio of four defense genes through qRT-PCR. The expression ratio of PR1 and thaumatin genes had FC value of 0.9 in the resistant line 72 h after infection, but the same genes had higher values in infected silks of the susceptible line (17.4 and 2.4 FC, respectively). On the contrary, the expression ratios of PR5 and PRm6 genes were 2.8 and 2.1 in CO441 and 6.0 and 38.8 in CO354, respectively, 72 h after *Fusarium* infection (Fig. 4.5 A). Induction of all four genes was low in the resistant line, with the lowest induction level for PR1 and thaumatin genes. As regards the expression ratio between the two lines before and 72 h after infection, PR1 and PR5 were over-expressed in the resistant genotype before *Fusarium* inoculation, while after the infection these two genes were more expressed in the susceptible genotype, as reported for seeds. PRm6 and thaumatin were up-regulated in CO354 both before and after infection (Fig. 4.5 B).

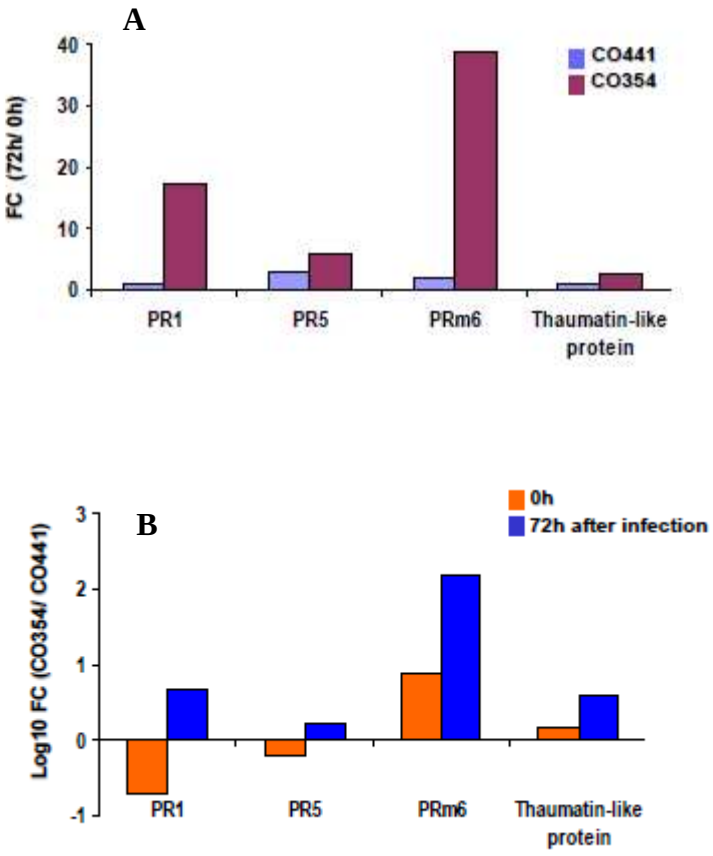


Fig. 4.5 A and **B** Fold change (FC) of differentially expressed genes in silks of CO441 and CO354 at 72 h after infection with Fusarium verticillioides, compared to 0 h. (B) Logarithm of FC of genes expressed before (0 h) and after (72 h) silks infection in CO354 compared to CO441.

4.2 Experiment 2

The aim of this analysis was to clarify the mechanisms of host response in the area surrounding the point of infection. Five stages of *F. verticillioides*-maize interactions were delineated: i) an early stage, at 12 and 24 h after infection, ii) an intermediate stage at 48 and 72 h after infection, and iii) a late stage at 96 h after infection. In addition, transcripts accumulation was studied in two host genotypes, infected with two fungal isolates.

4.2.1 Detection of wild type and mutant strains of *Fusarium verticillioides* in kernels

The growth of the wild type strain of *F. verticillioides* and its corresponding mutant *FUM*1^{-} was assayed by absolute quantification of the *tub* 2 gene through qRT-PCR. As showed in figure 4.6 A, in the infected kernels of the susceptible line CO354, the gene *tub* 2 of both strains was not present 12 h after infection. The gene was detectable starting from 24 h until 96 h after infection, with a higher copy number at 72 h (14100 copy number ± 141.4 *vs* 16500 copy number ± 707.2 for the wild type and *FUM*1^{-} strain, respectively). At 96 h after infection, the *tub* 2 copy number decreased in both fungi. Significant differences of *tub* 2 copy number were detected between the wild type and the mutant strains at 72 and 96 h post infection.

In the kernels of the resistant line CO441 infected with wild type and *FUM*1^{-}, the *tub* 2 copy number increased from 12 to 96 h after infection, but the presence of wild type strain was significantly higher respect to the mutant for each time of infection. The absolute number of copies was lower than that detected in CO354, as indicated in figure 4.6 B.

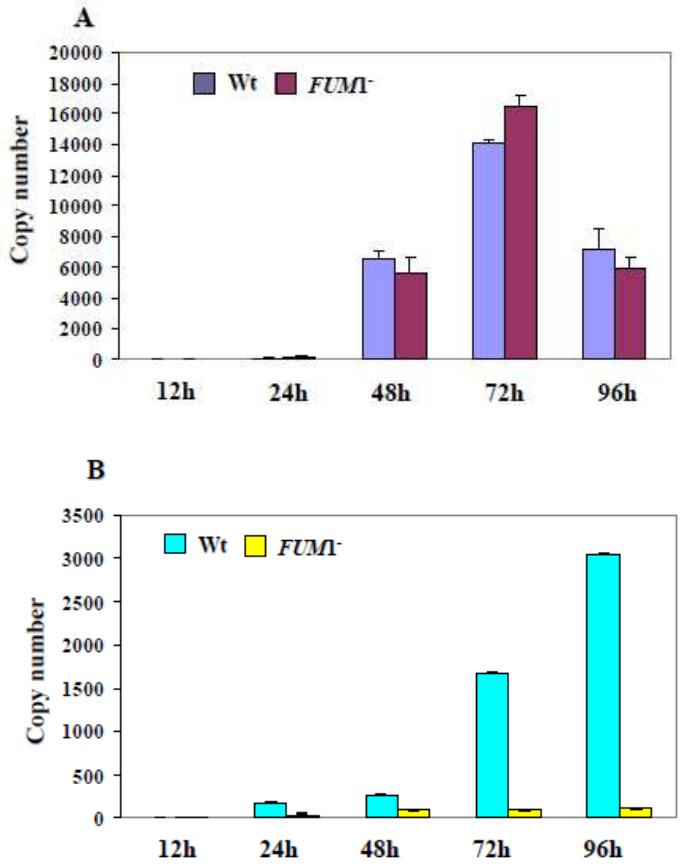


Fig. 4.6 A and **B** Copy number of the constitutive gene for *β-tubulin* 2 in kernels of the maize lines CO354 (A) and CO441 (B) infected with two strains of *F. verticillioides*, over a time course of 96 hours.

4.2.2 Gene expression analysis in kernels infected with wild type and mutant strains of *Fusarium verticillioides*

Since the absolute quantification of the *tub* 2 gene through qRT-PCR had displayed the absence of the wild type and *FUM*1^{-} strains for the time point 12 h, and a low copy number for the time point 24 h, around the point of infection in CO354 kernels, we analyzed gene expression data only for the comparisons between the control and 48, 72 and 96 h after infection. During the very early stages of infection (12 - 24 h) just a small proportion of the host transcripts was induced: 42 and 50 differentially expressed transcripts were recorded at 12 and 24 h post infection and none of them were involved in defense (data not shown). In this period the pathogen behaves like a biotroph (Bushnell *et al*., 2003) and the host has just begun to recognize that it is under attack. Between 48 and 72 h after infection the pathogen switches a necrotrophic mode of infection and the host recognizes infection has occurred and responds with a variety of defense strategies. As regards the wild type

strain of *F. verticillioides*, the total number of differentially expressed genes was 147 and 140 at 48 and 72 h after infection, respectively (Figure 4.7). At these two time points the total numbers of transcripts for host defense genes were constant (Table 4.5). At 48 h after infection a similar number of up- and down-regulated genes were found, while at 72 h after infection down-regulated genes prevailed.

The *FUM*1^{-} strain showed a different behaviour, because the plant responded with a higher number of total and defense genes (184 and 13 transcripts, respectively) at 72 h after infection, most of all were down-regulated (Figure 4.7 and Table 4.5).

Both the total number of differentially expressed genes and the number of host transcripts involved in defense were reduced at 96 h after infection, particularly in plants infected with the wild type strain (Figure 4.7 and Table 4.5).

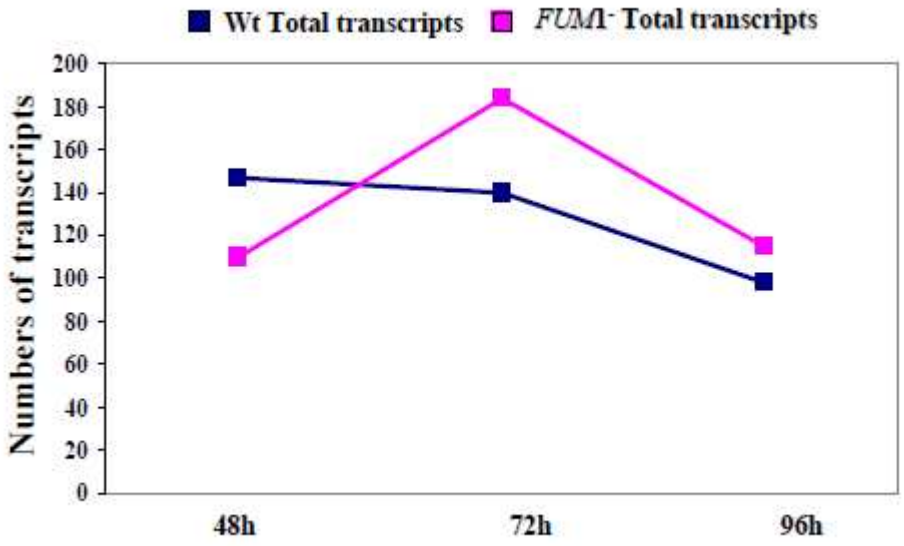


Fig. 4.7 Number of total host transcripts detected at three stages of infection with the wild type and *FUM*1^{-} strains of *F. verticillioides*.

Table 4.5 Number of total, up- and down-regulated defense transcripts detected at three stages of infection with wild type and *FUM*1^{-} strains of *F. verticillioides*.

Infection time/strain	Total number of defense genes	Up-regulated	Down-regulated
48 h Wt	11	6	5
72 h Wt	11	2	9
96 h Wt	6	3	3
48 h *FUM*1^{-}	7	2	5
72 h *FUM*1^{-}	13	2	11
96 h *FUM*1^{-}	11	3	8

4.2.3 Functional classes of genes expressed in kernels upon infection with wild type and mutant strains of *Fusarium verticillioides*

The differentially expressed genes in kernels upon infection with wild type and mutant strains of *F. verticillioides* were grouped according to the Munich Information Center for Protein Sequences (MIPS) FunCat database and classified in 12 functional categories as for the Experiment 1. Also in Experiment 2 the cellular functions with a lower number of differentially expressed genes were included in the functional class "Others" (Figures 4.8 and 4.9).

At 48 h after infection with the wild type strain, functional categories that were enriched in up- and down-regulated genes were: "Cell cycle and DNA processing", "Protein synthesis" and "Cell communication". The first category included 13.8% and 21.0% of up- and down-regulated genes, respectively; the second group comprised 11.2% and 12.0% and the third one 6.2% and 12.0% .The class "Cell rescue, defense and virulence" followed the above mentioned categories, with 7.5% of up- and down-regulated genes.

At 72 and 96 h post infection with the wild type strain, the most representative categories remained "Cell cycle and DNA processing" and "Protein synthesis". "Energy", "Cellular Communication" and "Cell rescue, defense and virulence" were also enriched with a high percentage of differentially expressed genes at 72 h after infection. At 96 h from infection the up- and down-regulated genes belonging to "Energy" class decreased, but the category "Metabolism" increased.

The main functional categories found at 48, 72 and 96 h post infection with the mutant strain were the same detected upon wild type infection, that were "Cell cycle and DNA processing" and "Protein synthesis". At 48 and 72 h post infection other two classes with several differentially expressed genes were involved in response to *FUM*1^{-} strain: "Cellular communication" and "Cell rescue, defense and virulence". This last category reached a high percentage (9.6%) of total transcipts at 96 h after mutant infection.

"Cell rescue, defense and virulence" was one of the prevalent functional classes, but it was not the group with the highest number of transcripts, unlike in Experiment 1, since kernels were sampled only in the area around the point of infection. Most of defense genes were down-regulated, expecially after *FUM*1^{-} strain attack, in support of the fact that probably the mutant *F. verticillioides* was less virulent and activated a blinder defense response in kernels. The most striking differences in the host response upon infection with wild type and mutant fungal strains were: i) higher

proportion (75%) of genes involved in defense were activated by the wild type isolate early during infection (48 h) and declined at later stage of infection (6.1% at 96 h); ii) 7% of defense responsive genes expression were induced by the mutant strain at a later time of infection (72 h), while 9.6% was induced at 96 h.

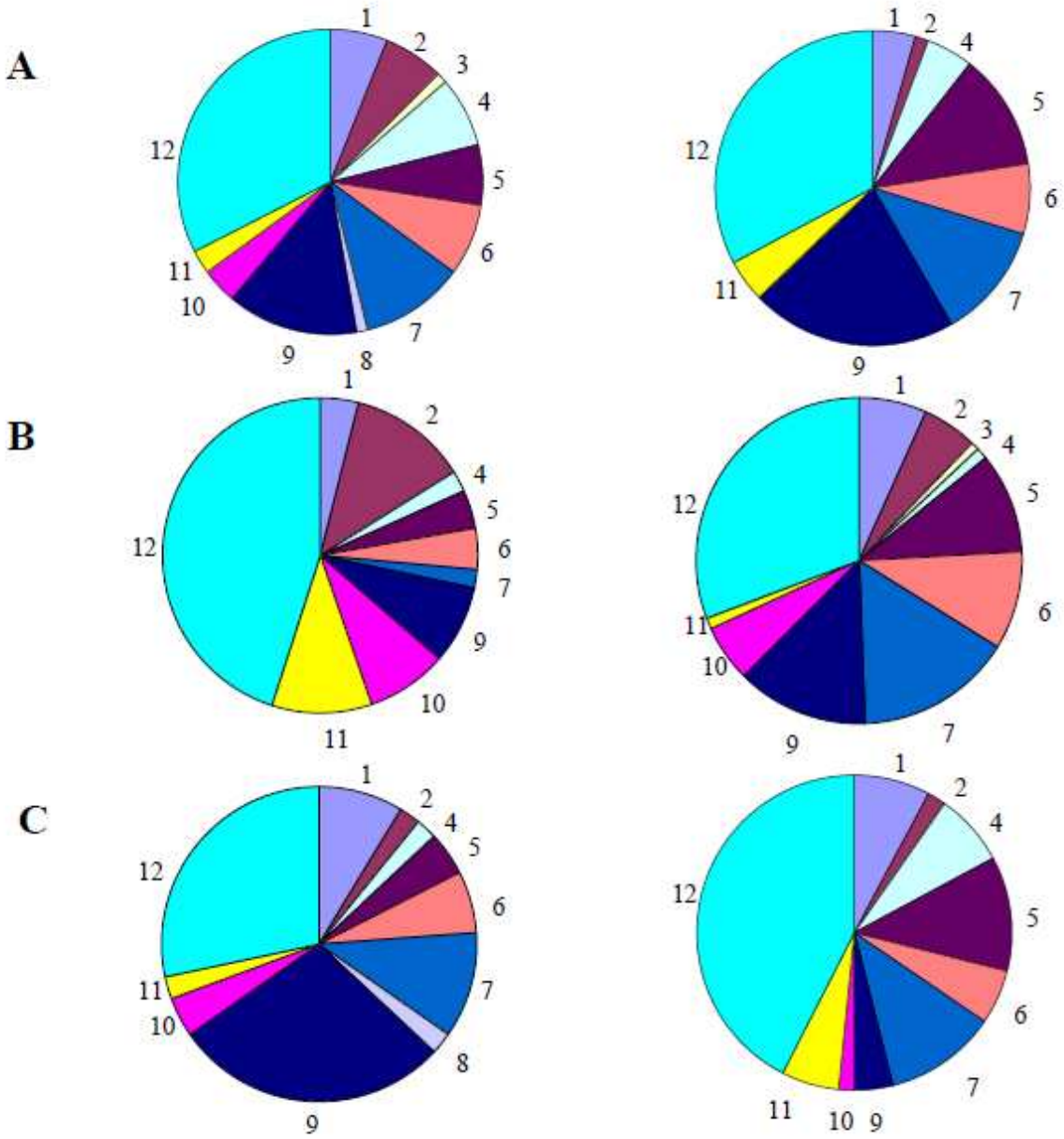


Fig. 4.8 Functional categories of up- (left pie chart) and down- (right pie chart) regulated genes found in CO354 upon 48 (A), 72 (B) and 96 (C) hours from infection with the wild type strain ITEM 10514 of *F. verticillioides*. Functional categories: 1)Metabolism; 2)Energy; 3)Development; 4)Cellular transport; 5)Cellular communication; 6)Cell rescue, defense and virulence; 7)Protein synthesis; 8)Storage protein; 9)Cell cycle and DNA processing; 10)Transcription; 11)Others; 12)Unknown.

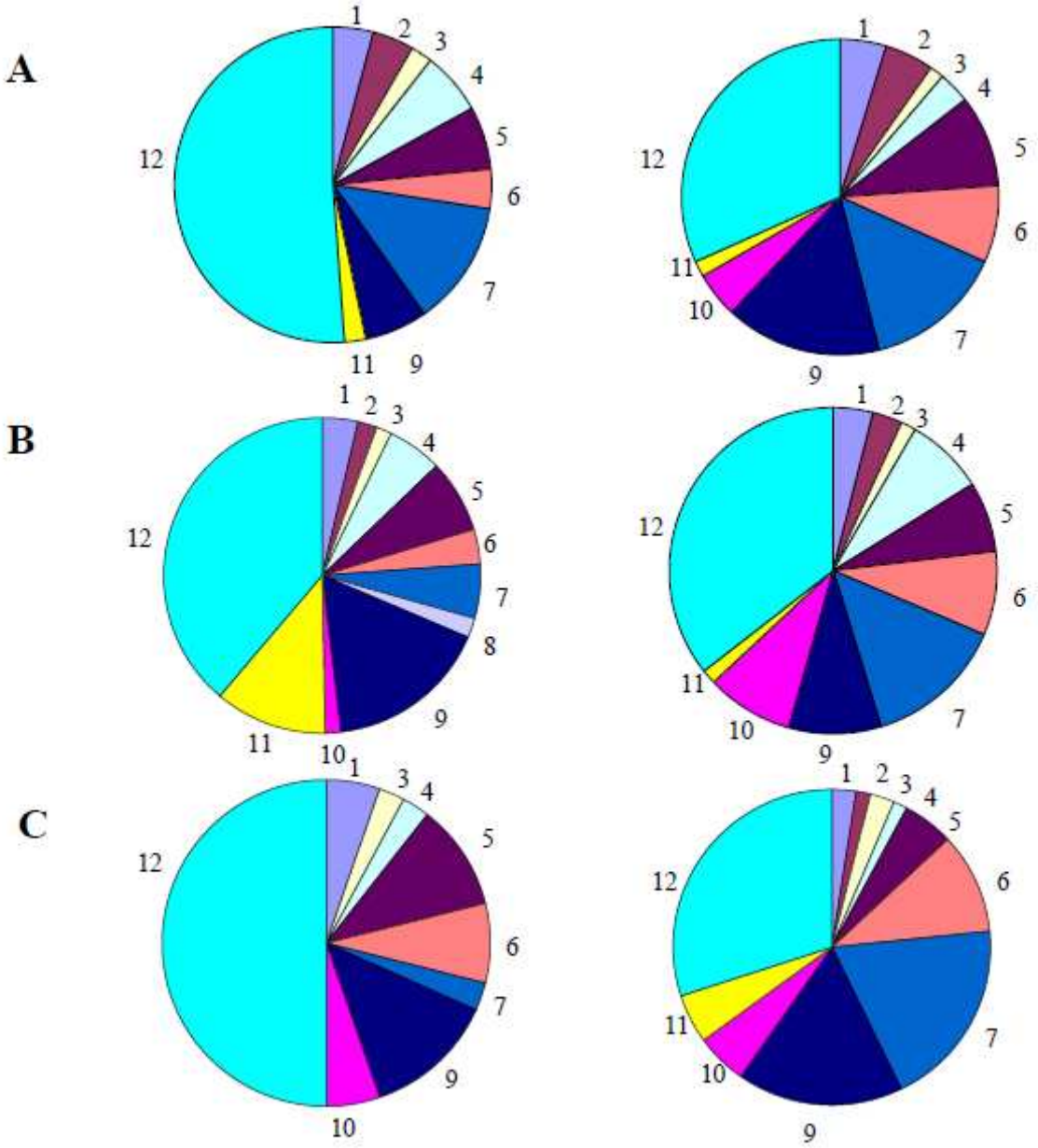


Fig. 4.9 Functional categories of up- (left pie chart) and down- (right pie chart) regulated genes found in CO354 upon 48 (A), 72 (B) and 96 (C) hours from infection with the mutant strain ITEM 10515 of *F. verticillioides*. Functional categories: 1)Metabolism; 2)Energy; 3)Development; 4)Cellular transport; 5)Cellular communication; 6)Cell rescue, defense and virulence; 7)Protein synthesis; 8)Storage protein; 9)Cell cycle and DNA processing; 10)Transcription; 11)Others; 12)Unknown.

4.2.4 Maize kernels exhibited multiple responses to infection by wild type and mutant strains of *Fusarium verticillioides*

Among all differentially regulated genes, of particular interest was the identification of a large number of genes that encoded proteins involved in signal transduction and regulation of gene expression. These included five phosphatates, twenty two protein kinases, four calcium-binding proteins, two GTP-binding proteins and seventeen transcription factors (Table 4.6). In the protein kinase group, several plant-specific protein kinases, including the family of receptor-like kinases (RLKs, BM333923 and CD527568), were found to be regulated after both wild type and mutant strain of *F. verticillioides* infection. RLKs are believed to perceive external stimuli and transduce the signals across the plasma membrane through phosphorylation cascades that ultimately led to expression of appropriate target genes. During the activation of plant defense responses, pathogen- or host-derived elicitor molecules may serve as ligands for these RLKs (Song *et al*., 1995).

Genes for Ca^{++}-binding proteins were transcriptionally activated by infection with the two strains of *F. verticillioides*, including calmodulin (CD651384 and DV551099) and calcineurin B protein (DV489710). These proteins are calcium sensors that specify the cellular response to different extracellular signals (Kudla *et al.*, 1999).

GTPases, as ADP-ribosylation factor (ARF) GTPase (DV621283), rac GTPase activating protein 3-like protein (DV492869) and ran GTPase binding (CB603999) were also differentially expressed. A recent work from Böhlenius *et al*. (2010) explores the role of host cell vesicle transport in barley (*Hordeum vulgare*) resistance against the podwery mildew fungus *Blumeria graminis* f. sp *hordei* (*Bgh*). The ADP-ribosylation factor (ARF) GTPases were involved in vesicle budding and implicated in resistance to *Bgh* penetration in barley leaves.

The expression of a large number of genes encoding transcription factors were also detected, in particular members of WRKY and MYB families. WRKY1 (CB381418 and CB617138) and WRKY-like DNA-binding protein (CB834015) were differentially expressed. Different WRKY transcriptional factors showed diverse expression patterns following infection. WRKY1 was up-regulated, whereas WRKY-like was down-regulated by infection with both wild and mutant strains of *F.verticillioides*. WRKY proteins were originally defined as transcription factors that target genes regulated by pathogen attack, fungal elicitors and SA (Rushton *et al.*, 1996). Many genes are repressed by WRKY factors bound to their promoters

(Rushton *et al.*, 2010). WRKY proteins are central components of many aspects of basal defense system. The presence of WRKY genes reported in this work supports the notion that WRKY is involved in maize defense mechanisms against *F. verticillioides*.

Infection altered also the gene expression of MYB family transcription factors (CB815822 and CB834037)). The NtMYB2 gene from tobacco (*Nicotiana tabacum*) was found to be inducible by wounding and elicitors and is known to positively regulate PR gene expression (Sugimoto *et al*., 2000). Therefore other MYBs, like AtMYB15 and AtMYB51 were found up-regulated. The up-regulation of MYB transcription factors could support the hypothesis that *F. verticillioides* infection positively regulates the secondary metabolic pathways involved in the activation of defense response.

Several genes encoding putative components of disease resistance pathways were also shown to be activated after wild type and mutant strains of *F. verticillioides* infection. These genes included: a pathogenesis related protein-5 (BM073434); a hypersensitive-induced response protein (DV943246), a positive regulator of hypersensitive cell death in plants (Jung *et al*., 2008) and a PDR6 ABC transporter (DV491613), that plays a role in the pathogen reaction since it has been shown that plant pathogenic fungi need the activity of PDR-like genes for the expression of pathogenicity or acquired resistance towards fungicide (Martinoia *et al*., 2002).

The results also confirmed that the oxidative burst, an essential component of the pathogen response, was highly regulated after pathogen attack. Some genes for the enzymes linked to oxidative stress were activated, such as: glutathione S-transferase (GST 11, BM337569); glutathione S-transferase (GST 12, BM078815); NADH-ubiquinone oxidoreductase (DV549936); aldehyde dehydrogenase (DV550950).

Many differentially expressed genes found in Experiment 2 were involved in secondary metabolism and cell wall modifications. Relevant is the activation of the phenylpropanoid pathway that produces many secondary metabolites, which act as anti-pathogen and anti-insect agents (De Luca and St-Pierre, 2000), like the enzyme cinnamoyl-CoA reductase (DV549841). Genes that partecipate in cell wall biosynthesis and modifications, listed in Table 4.6, are known to be regulated after pathogen infections (Maleck *et al.*, 1999; Schenk *et al.*, 2000). We found cellulose synthase-2 (DV621987), expansins (DV495624, DV551178), glycosyl transferases (CA829522, CB381353), which alter carbohydrate bonds and modify secreted cell wall components.

Heat shock proteins (HSPs) play an essential part in protein folding and other cellular processes under normal and stress conditions in plants (Lee and Vierling, 2000). *F. verticillioides* infection activated several genes encoding HSPs, like Cytosolic HSP90 (DV495478), HSP82 (DV493376), HSP binding (DV550016) and HSP90-2 like (CD527681).

Many genes involved in auxin signaling pathway were surprisingly detected, as for example: an auxin binding protein (DV622250); three auxin response factors (BM335723, DV550009, CB329403); two GH1 auxin-regulated proteins (CD484260, Dv490896). Although auxins, primarly indole-3-acetic acid (IAA), control many important developmental processes in plant (Normanly and Bartel, 1999), little is known on auxin function in stress and defense responses.

Table 4.6 Differentially expressed genes encoded upon wild type and *FUM*1$^-$ strains of *F. verticillioides* infection

Gene Family	ID	p value	Infection time /strain
Phosphatases			
Phosphatase 2A regulatory subunit A	DV490818	*; ***	72 h Wt; 72 h *FUM*1$^-$
Protein phosphatase 2C	DV491349	*	72 h Wt
Putative phosphatase	CA829703	*	48 h *FUM*1$^-$
Tyrosine specific protein phosphatase-like	BG841147	*; *	72 h Wt; 72 h *FUM*1$^-$
Tyrosine specific protein phosphatase-like	CB603810	*	48 h *FUM*1$^-$
Protein kinases			
Casein kinase	CB833634	*	72 h *FUM*1$^-$
Casein kinase II alpha subunit	DV495959	**; ***	72 h *FUM*1$^-$; 96 h *FUM*1$^-$
Cyclin-dependent kinases regulatory subunit	BG873914	*	72 h *FUM*1$^-$
Histidine kinase 3	DV549613	*	48 h *FUM*1$^-$
Leucine-rich repeat transmembrane protein kinase 1	CB250095	*	72 h *FUM*1$^-$
Mitogen-activated protein kinase	DV549714	**	96 h Wt
Protein cdc2 kinase	CB381548	*	48 h *FUM*1$^-$
Protein kinase	CA989188	*	48 h Wt
Protein kinase	CB605069	*	48 h Wt
Protein kinase	DV489876	*	48 h Wt
Protein kinase	DV492782	*	48 h *FUM*1$^-$
Protein kinase	DV495048	*	72 h Wt
Protein kinase-like	CB381553	*;*;*	48 h Wt; 48 h *FUM*1$^-$; 96 h *FUM*1$^-$
Protein kinase-like	DV492677	*	48 h Wt
Protein kinase ZmMEK1	DV494772	**	96 h Wt
Receptor kinase	CD527568	**;***	72 h Wt; 72 h *FUM*1$^-$
Receptor-like kinase homolog	BM333923	***	48 h Wt;
Serine/threonine kinase	CB251893	**	72 h *FUM*1$^-$
Ser-thr protein kinase	BQ539488	*	72 h Wt
Shaggy-like kinase dzeta	DV492995	**	96 h *FUM*1$^-$
Sphingosine kinase	DV621841	***; *; **; ***	48 h Wt; 72 h Wt; 72 h *FUM*1$^-$; 96 h *FUM*1$^-$

Table 4.6 Continued 1

Wall-associated serine/threonine kinase	DV491303	*	72 h Wt
Calcium-binding proteins			
Calcineurin B protein	DV489710	**	48 h Wt
Calcium-binding protein	CD651345	**	96 h Wt
Calmodulin binding	CD651384	**	72 h *FUM*1$^-$
Calmodulin cam-207	DV551099	***	96 h Wt
GTP-binding proteins			
ADP-ribosylation factor (ARF) GTPase	DV621283	***	48 h Wt
ATARLA1D GTP binding	DV942623	**	48 h *FUM*1$^-$
Rac GTPase activating protein 3-like protein	DV492869	***	48 h *FUM*1$^-$
Ran GTPase binding	CB603999	***	72 h *FUM*1$^-$
Ras-related GTP-binding protein	DV942952	***	96 h Wt
Transcription factors			
Homeobox transcription factor KNOTTED1	DQ056233	**	96 h Wt
LIM transcription factor homolog	CB381016	***; **; ***	72 h Wt; 96 h Wt; 96 h *FUM*1$^-$
LIM transcription factor homolog	DV621117	**	72 h *FUM*1$^-$
MADS-domain transcription factor	BM379590	**	96 h *FUM*1$^-$
Myb family transcription factor	CB815822	**	72 h *FUM*1$^-$
MYB-like DNA binding protein	CB834037	**	96 h Wt
OCL4 protein	CD651585	**	48 h Wt
RING-H2 zinc finger protein	CB351502	**	72 h *FUM*1$^-$
Tubby protein	CA829576	**; ***	72 h Wt; 72 h *FUM*1$^-$
Zinc finger protein	CB816146	**	72 h *FUM*1$^-$
Zinc finger protein	DV495481	**	72 h Wt
Zinc finger protein-like	CD573376	***	48 h Wt
Zinc finger protein 3-like	CB834000	***; ***;***; ***	48 h *FUM*1$^-$; 72 h Wt; 72 h *FUM*1$^-$; 96 h *FUM*1$^-$
Zinc finger (C3HC4-type RING finger)-like protein	CD527890	**	72 h *FUM*1$^-$
Zinc finger (C3HC4-type RING finger)-like protein	DV494415	**	96 h Wt
Zinc finger (C3HC4-type RING finger)-like protein	DV622592	**	72 h *FUM*1$^-$
WRKY-like DNA-binding protein	CB834015	***; **; ***	48 h *FUM*1$^-$; 72h Wt; 72h *FUM*1$^-$

Table 4.6 Continued 2

WRKY1	CB617138	***; ***	48 h Wt; 96 h Wt
WRKY1	CB381418	**; ***; **; ***	48 h Wt; 48 h *FUM*1$^-$; 72 h Wt; 96 h *FUM*1$^-$
Pathogenesis-related genes			
Cf-5 disease resistance protein-like	CB815940	**	48 h Wt
Endo-1,3(4)-beta-glucanase	DV621703	**	96 h *FUM*1$^-$
Jacalin	CD651123	**; ***; ***; ***; ***	48 h *FUM*1$^-$; 72 h Wt; 72 h *FUM*1$^-$;96 h Wt; 96 h *FUM*1$^-$
Hypersensitive-induced response protein	DV943246	***	96 h Wt
Interferon-related protein	DV492114	**	96 h Wt
MtN19	BG840538	**; ***	72 h *FUM*1$^-$; 96 h *FUM*1$^-$
Pathogenesis-related protein 5	BM073434	**	96 h Wt
PDR6 ABC-transporter	DV491613	**	96 h Wt
Serine protease inhibitor	DV495714	***	96 h *FUM*1$^-$
TMV-MP30 binding protein 2C-like	CD485178	**	96 h Wt
Oxidative Burst			
Acyl-CoA oxidase	CD527905	**	72 h *FUM*1$^-$
Aldehyde dehydrogenase	DV550950	**; ***	72 h *FUM*1$^-$; 96 h *FUM*1$^-$
Aldehyde dehydrogenase RF2B	CB380932	***	72 h *FUM*1$^-$
Cytochrome c	BM080612	**	72 h *FUM*1$^-$
Ferredoxin-3	DV493762	**; ***; **	48 h *FUM*1$^-$; 72 h Wt; 96 h *FUM*1$^-$
Glutathione peroxidase	DN210036	**; ***	72 h Wt ; 72 h *FUM*1$^-$
Glutathione S-transferase GST 11	BM337569	***; *; ***; ***	48 h Wt; 48 h *FUM*1$^-$; 72 h Wt; 96 h *FUM*1$^-$
Glutathione S-transferase GST 12	BM078815	*	48 h Wt
Manganese superoxide dismutase SOD-3	CB616954	***	48 h Wt
Mn-superoxide dismutase	DV495005	***; ***	72 h Wt ; 72 h *FUM*1$^-$
NADH-ubiquinone oxidoreductase	DV549936	**	48 h Wt
Pyridoxamine 5-phosphate oxidase	DV621295	***; ***	72 h *FUM*1$^-$; 96 h Wt
Superoxide dismutase-4AP	CD484410	***	72 h *FUM*1$^-$
Phenylpropanoid biosynthesis			
Cinnamoyl-CoA reductase	DV549841	***	72 h *FUM*1$^-$
Cell wall modification			

Table 4.6 Continued 3

α-expansin 2	DV495624	**	72 h Wt
β-expansin	DV551178	***	96 h Wt
Beta 1,3-glycosyltransferase-like protein II	CA829522	***;***	48 h Wt; 48 h *FUM*1$^-$
Caffeoyl CoA 3-O-methyltransferase	BM350128	**	96 h Wt
Cellulose synthase-2	DV621987	**	48 h Wt
Sucrose-UDP glucosyltransferase 2	CB381353	**	96 h *FUM*1$^-$
Heat shock responsive proteins			
Cytosolic heat shock protein 90	DV495478	**	48 h Wt
Heat shock protein binding	DV550016	**	72 h *FUM*1$^-$
Heat shock protein 82	DV493376	**	48 h *FUM*1$^-$
Hsp90-2-like	CD527681	***; ***; ***	72 h Wt; 72 h *FUM*1$^-$; 96 h *FUM*1$^-$
Auxin pathway			
Auxin-binding protein	DV622250	**	48 h Wt
Auxin response factor	BM335723	**	72 h *FUM*1$^-$
Auxin response factor 7b	DV550009	**	48 h Wt
Auxin response transcription factor (ARF6)	CB329403	**	48 h *FUM*1$^-$
GH1 protein or auxin-regulated protein	CD484260	**	72 h Wt
GH1 protein or auxin-regulated protein	DV490896	**	48 h Wt

*$p<0.01$; **$p<0.005$; ***$p<0.001$.

4.2.5 qRT-PCR of selected marker genes in kernels infected by wild type and mutant strains of *Fusarium verticillioides*

To validate the microarray results, eight differentially expressed genes were chosen and confirmed by qRT-PCR, together with seven genes detected in Experiment 1, that included peroxidase, thaumatin, WIP1, PRm6, burst-oxidase protein, β-glucosidase and chitinase PRm3. The selected genes were assayed in CO354 kernels at 24, 48, 72 and 96 h after wild type and *FUM*1^{-} strains of *F. verticillioides* (Figure 4.10). The expression patterns of these genes fitted with microarray experiments: for example, in the microarray WRKY1 (CB617138) had fold changes of -3.2 and -4.0 in kernels infected at 48 and 96 h with wild type strain, respectively, and in qRT-PCR, the FCs were -3.3 and -1.0 in kernels at the same times of infection. MYB-like DNA binding protein (CB834037) had FCs of 1.5 and 2.3 in kernels infected at 96 h with wild type strain with array and qRT-PCR respectively. G3PDH (CB816042) was down-regulated both in array (FC -1.5) and qRT-PCR (FC -1.8) analysis in kernels at 96 h after infection with the wild type strain. The qRT-PCR expression trends of the remaining five genes (PDR 6 ABC transporter, β-expansin 1, hypersensitive induced response protein, PR5 and calcineurin B protein) were almost consistent with the results obtained from the microarray analysis.

To examine the functions of the differentially expressed genes, they were sorted into different expression patterns induced by the wild type fungal strain (Figure 4.10).

The first pattern showed a peak expression at 72 h and a drop thereafter. This group included most of the up-regulated genes related to defense, such as PR5, PRm6, peroxidase, thaumatin, Bowman-Birk proteinase inhibitor WIP1 and chitinase PRm3. G3PDH, involved in sugar metabolism, and β-expansin1, related to cell wall modification, had the same expression pattern, but they were down-regulated between 24 and 48 h.

The gene expression in the second group showed an increasing trend at transcipts level with expression peak at 96 h after infection. The genes in this group were related to defense (PDR6 ABC transporter, hypersensitive induced response protein, β-glucosidase) and transcriptional regulation (WRKY and MYB). The expression of Calcineurin B protein reached the lowest level at 24 and 72 h, whereas there was no dramatic transcription changes in the other two infection stages. Finally, burst-oxidase protein was expressed similarly during the entire expression process.

The most notable features induced by the mutant *Fusarium* strain were that, in the first group, PR5, peroxidase and Bowman-Birk gene expression were down-regulated between 48 and 72 h. The β-expansin was found up-regulated at the times of infection where the wild type was down-regulated. The G3PDH started to be up-regulated at 24 h and was down-regulated thereafter. In our results the PDR6 ABC transporter was constantly up-regulated after 48 h. The transcriptional regulator WRKY was differentially under-expressed only at 24 and 96 h. The level of Calcineurin transcripts was evidently up-regulated since 24 h.

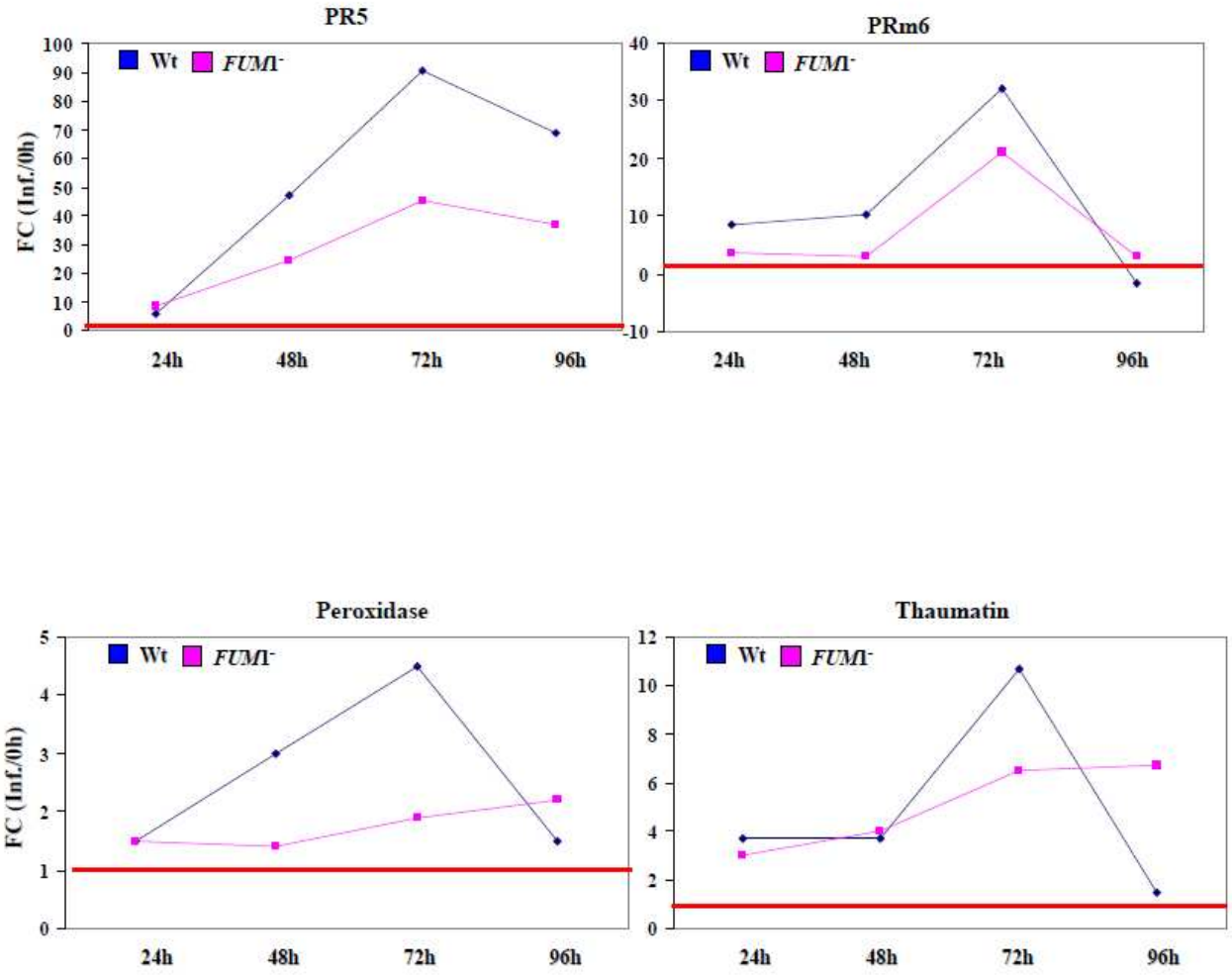


Fig. 4.10 Fold change (FC) of differentially expressed genes in kernels of CO354 line at 24, 48, 72 and 96 h after infection with wild type (blue line) and *FUM*1^{-} (pink line) strains of *F. verticillioides*. The red line represents FC=1, indicating no difference in expression between infected and not inoculated kernels.

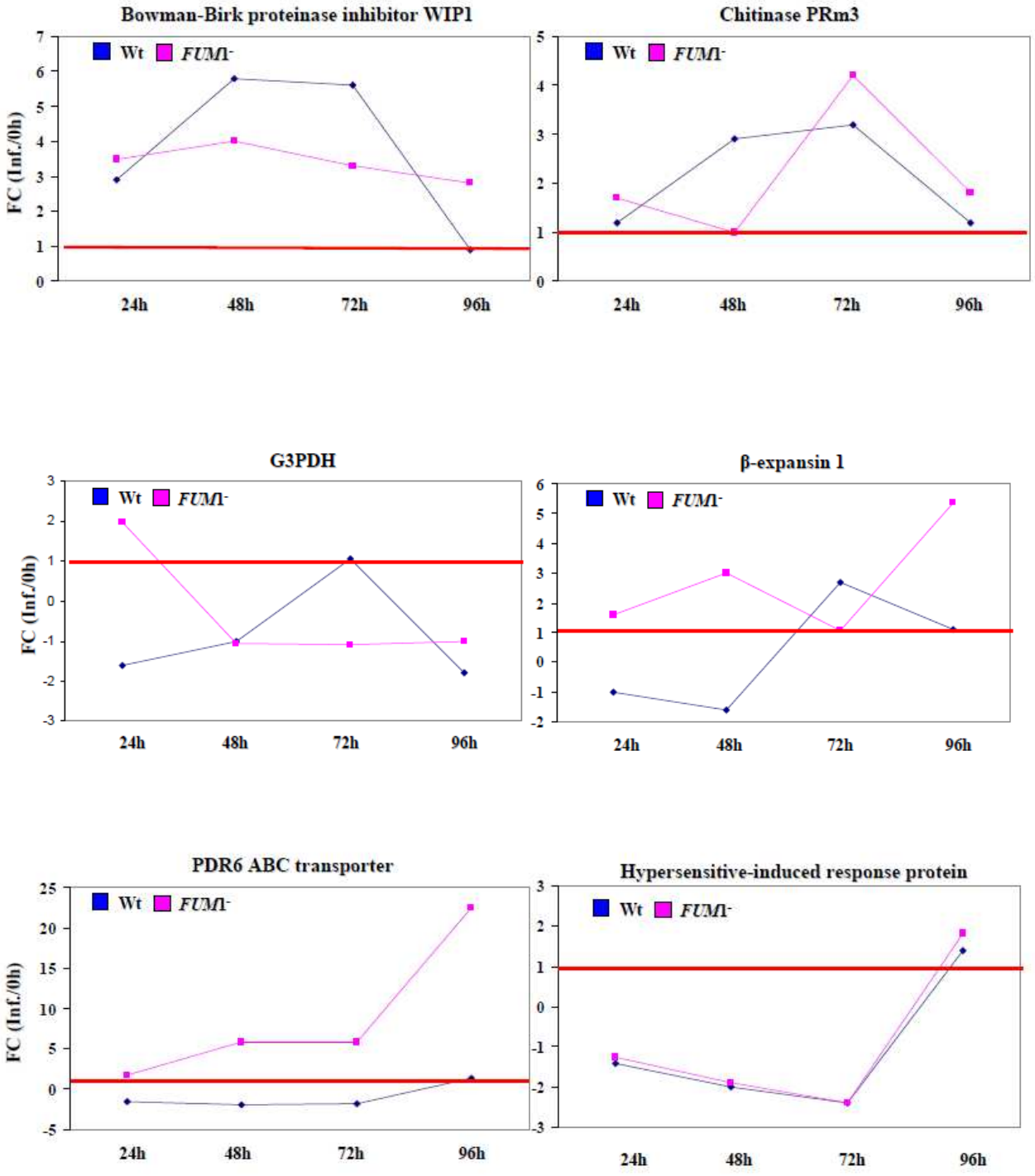


Fig. 4.10 Continued 1.

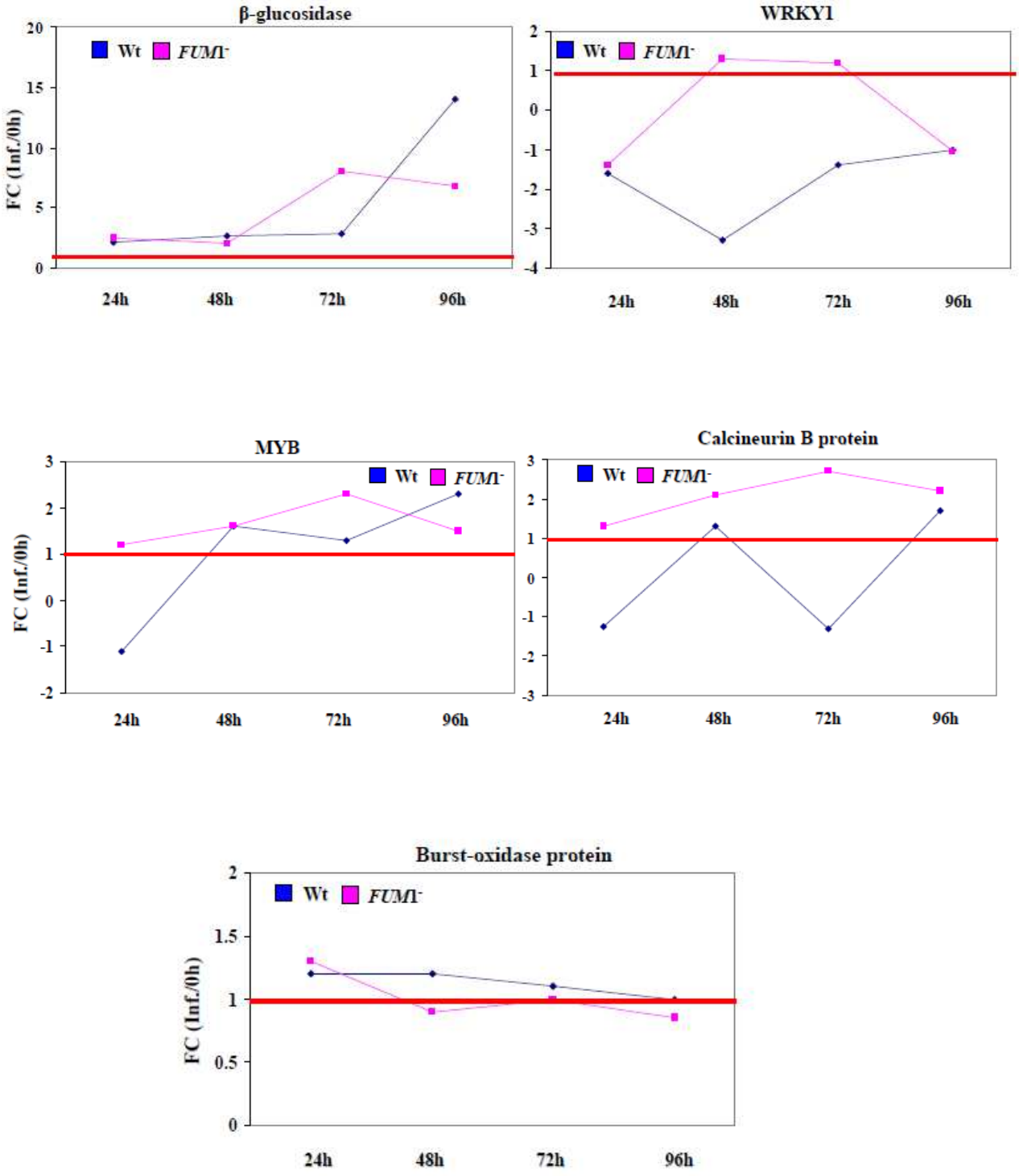


Fig. 4.10 Continued 2.

Ten out of the fifteen differentially expressed genes were tested in CO441 kernels at 48 and 72 h after infection with the wild type and *FUM*1^{-} strains of *F. verticillioides* (Figure 4.11). Results showed that five genes (PR5, PRm6, Bowman-Birk proteinase inhibitor WIP1, β-glucosidase and Chitinase PRm3) exhibited increase transcripts accumulation in resistant kernels infected with both *F. verticilllioides* strains in a time

period of 24 h compared with the susceptible ones. These evidences were apparently in contrast with data obtained in Experiment 1, where the expression levels of these genes were higher in the susceptible line 48 h after infection. The infection may elicits the activation of signaling pathways that coordinate the resistance to pathogen in tissues far from the point of penetration. The level of gene activation is supposed to be lower in this region respect to the starting point of the infection (compare Figures 4.11 and 4.4 A). The resistant line may activate a more efficently battery of defense genes, before the invasion by the fungus in non damaged tissues or in adjacent ones. Several data documented that a defense response can be elicited in maize seeds and seedlings by endogenous elicitors, such as SA, JA or ethylen (Rajjou *et al.*, 2006).

There were few exceptions represented by the burst-oxidase protein and peroxidase, whose expression was in agreement with Experiment 1. WRKY and G3PDH genes remained substantially down-regulated in both maize genotypes. A strong induction of thaumatin expression was observed at 48 h post infection with the mutant strain.

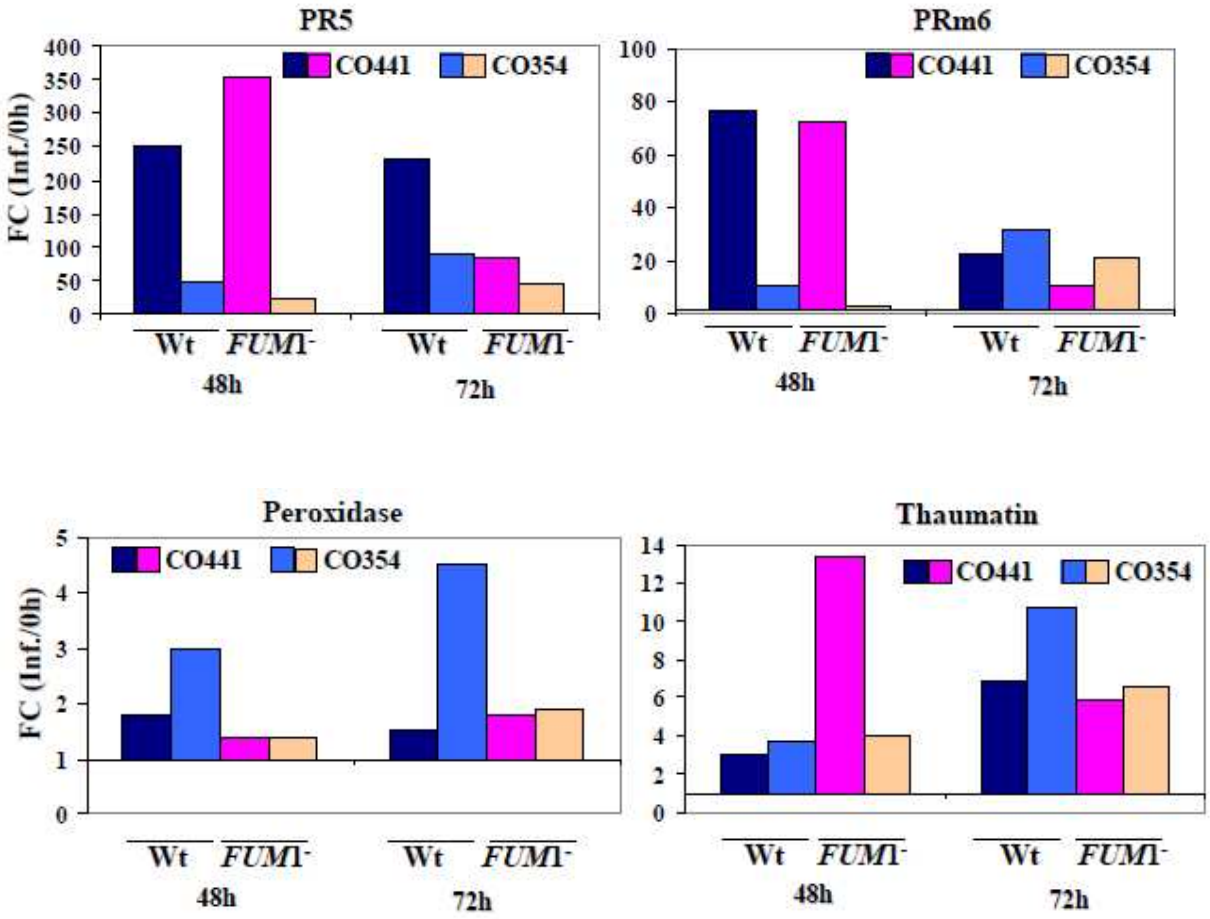


Fig. 4.11 Fold change (FC) of differentially expressed genes in kernels of CO441 line at 48 and 72 h after infection with wild type and *FUM*1^{-} strains of *F. verticillioides*.

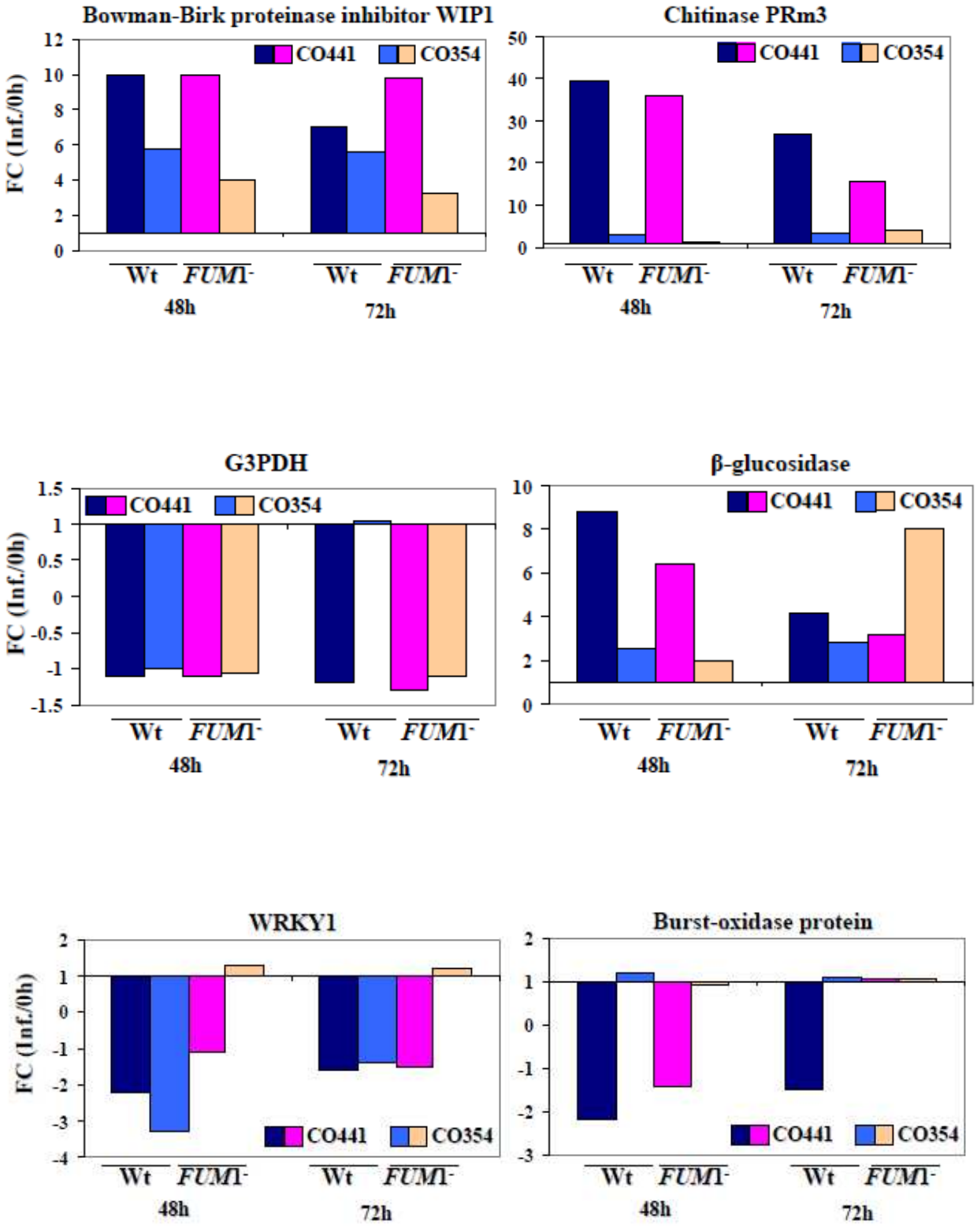


Fig. 4.11 Continued.

4.3 Experiment 3

The differential expression of pathogen-related genes between resistant and susceptible genotypes were decribed in the previous two experiments. The results obtained indicated that a basal response inhibited *F. verticillioides* spread in the resistant genotype. These observations prompted to investigate the effect of infection by different fungi on defense genes in maize genotypes with contrasting levels of resistance.

4.3.1 Genotype response to *Fusarium verticillioides* infection

In addition to CO354 and CO441 lines, several inbreds were evaluated for *Fusarium* ear rot resistance. Inbred lines CO387, CO430, CO441 and GT-MAS:gk showed a rating of resistance to *Fusarium* ear rot and a percentage of infected kernels lower compared to the other lines. CO431, CO432, CO433 displayed intermediate values of resistance, while CO354, CO388 and CO389 turned out to be the inbreds with the highest ear rot severity and incidence of infected kernels. It was noticed that the inbred lines with higher susceptibility to *Fusarium* ear rot (CO354, CO388 and CO389) had a later flowering time respect to the resistant and intermediate lines (Table 4.7). The correlation between percentage of *Fusarium* ear rot and flowering time was high (r = 0.88).

Table 4.7 Rating and percentage of *Fusarium* ear rot in ten maize inbred after two years testing.

Genotype	*Fusarium* ear rot		Flowering time (day from sowing)
	Rate (1-6)	Percentage	
CO354	4.0	80.0	81
CO387	2.6	16.0	69
CO388	4.2	70.0	79
CO389	4.2	90.0	79
CO430	2.5	10.0	68
CO431	3.4	40.0	68
CO432	3.2	40.0	69
CO433	3.6	45.0	69
CO441	2.4	16.0	69
GT-MAS:gk	2.4	15.0	70
L.S.D. (0.05)	*0.3*	*8.0*	*1*

4.3.2 Preliminary investigations on basal defense against several pathogen species

With the aim to reveal mechanisms that maize kernels develop against *F. verticillioides*, the *PRm*6 was chosen as one of the most abundant transcripts that accumulated in infected tissues of resistant and susceptible genotypes. *PRm*6 belongs to the maize PR proteins with 1,3-β-glucanase activity and its role in defense is to restrict the growth of the fungus by cleaving 1,3-β-glucans contained in the pathogen cell walls. The expression of *PRm*6 gene was investigated in CO354 and CO441 kernels 72 h after infection with the following fungal species: *F. verticillioides, A. flavus, F. subglutinans* and *F. proliferatum*, through qRT-PCR (Figure 4.12). In this study the accumulation of *PRm*6 transcript was induced after fungal infection in both maize lines. Although differences in the FC expression values of *PRm*6 gene were present among different species used, all the fungi assayed were able to induce *PRm*6 expression. As expected, pathogen-induced accumulation of *PRm*6 reached higher levels in CO354 than CO441 at 72 h after infection (up to 1400 fold in average). Our results were in agreement with those

reported by Murillo *et al.* (1999), showing that seven fungi were able to induce PR1 accumulation. These data suggested that accumulation of PR1 could be a general response of germinating maize seeds to fungal infection.

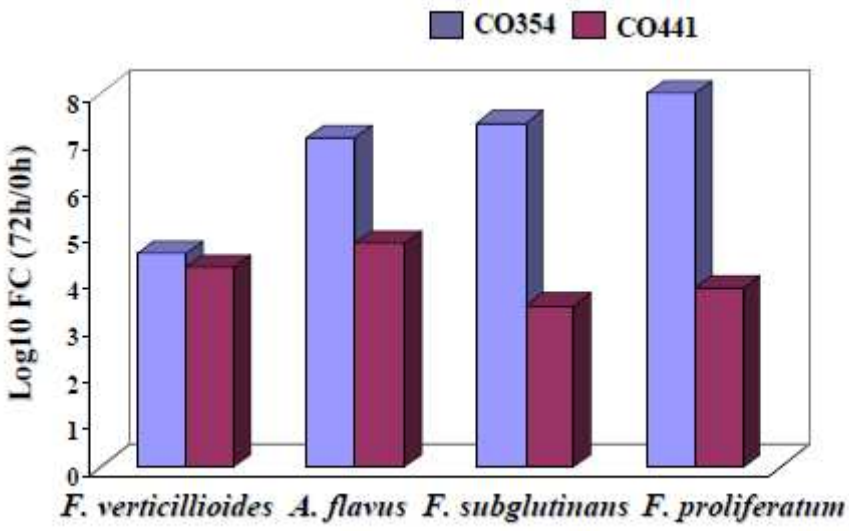


Fig. 4.12 Logarithm of fold change (FC) of *PRm*6 gene in kernels of CO354 and CO441 at 72 h after infection with different fungal species, compared to 0h.

4.3.3 Basal defense response in maize genotypes with different levels of resistance to *Fusarium verticillioides*

The expression of *PRm*6 gene was tested in developing kernels belonging to several resistant (R), susceptible (S) and intermediate (I) genotypes: CO354 (S), CO387 (R), CO388 (S), CO389 (S), CO430 (R), CO431 (I), CO432 (I), CO433 (I), CO441 (R) and GT-MAS:gk (R) (Table 4.8).

At 72 h after infection with wild type strain of *F. verticillioides*, *PRm*6 was highly up-regulated in all genotypes analyzed. The level of induction was stronger in susceptible lines, followed by intermediate lines, while resistant lines responded with lower FC values. FC values of susceptible and intermediate genotypes matched with agronomic *Fusarium* ear rot resistance data, because the *PRm*6 expression was correlated with ear rot percentage (r = 0.71). Among the resistant lines, the low incidence of ear rot in CO441 was associated with a higher induction level of *PRm*6 expression.

The results of this experiment confirmed that susceptible genotypes respond specifically to pathogen infection, while resistant genotypes has already high levels

of defense-related transcripts before infection, that provide a basal defense system to different fungal species. Although these data refer to the expression of one defense gene, they are very promising for further expression analysis of additional pathogen related genes.

Table 4.8 Fold change (± standard deviation) of *PRm*6 expression in kernels of different resistant (R), susceptible (S) and intermediate (I) genotypes at 72 h after infection with *F. verticillioides*.

Genotype	**FC value ± SD**
CO354 (S)	40350 ± 1186
CO387 (R)	2452 ± 48
CO388 (S)	163338 ± 13867
CO389 (S)	549370 ± 135911
CO430 (R)	543 ± 18
CO431 (I)	24780 ± 1820
CO432 (I)	22931 ± 337
CO433 (I)	39794 ± 1170
CO441 (R)	21494 ± 1474
GT-MAS:gk (R)	641 ± 34

Chapter 5

Conclusions

Plants are constantly subjected to infection by a variety of microbial pathogens, but a battery of defense mechanisms for contrasting microbial diseases was developed through evolution. Upon infection by a microbial pathogen, a resistant plant is often able to recognize the invading pathogen through the specific interaction between pathogen encoded molecules, called elicitors, and plant host receptors (many of which may be encoded by disease resistance genes). Subsequent signal transduction events can be triggered and they activate a series of defense responses, including induction of defense-related genes (Yang *et al*., 1997).

In this study we investigated global gene expression at several time points after different fungal species infection. In the first part of the work resistant and susceptible genotypes to *Fusarium* ear rot were tested 48 h after infection with a wild type strain of *F. verticillioides*. Kernels and silks of the two maize lines infected, sampled in the point of infection, presented high level of variability in response to *F. verticillioides* infection. Similar functional categories of genes were involved in response to infection in both resistant and susceptible lines and the most relevant class was “Cell rescue, defense and virulence”. In the resistant genotype most of the genes were already made transcripts (quantitative class) and provided a basal level of defense to the fungus. Instead, in the susceptible genotype most of the genes were qualitatively induced from a basal level and responded specifically to pathogen infection.

In the second part of the work the expression analysis was extended to early and late phases of infection (from 12 to 96 hours after infection) and kernels were sampled in the area around the point of infection to evaluate the diffusion of wild type and mutant strains of *F. verticillioides*. Most of genes were differentially regulated 48 h after infection with both fungal strains. The wild type strain was able to activate host defense genes before the mutant strain. For some genes, the resistant line had a higher level of expression than the suceptible line, since having analyzed the area around the point of infection the resistant genotype had already activated defense mechanisms. All differentially expressed genes detected in our experiments were distinguished in early response genes, that encode “signalling or regulatory components”, such as protein kinases and transcription factors, and late response genes, that encode “effector proteins” such as enzymes involved in metabolism. In

several cases products of early response genes regulated the expression of late response genes, suggesting a cascade of regulation (Figure 5.1).

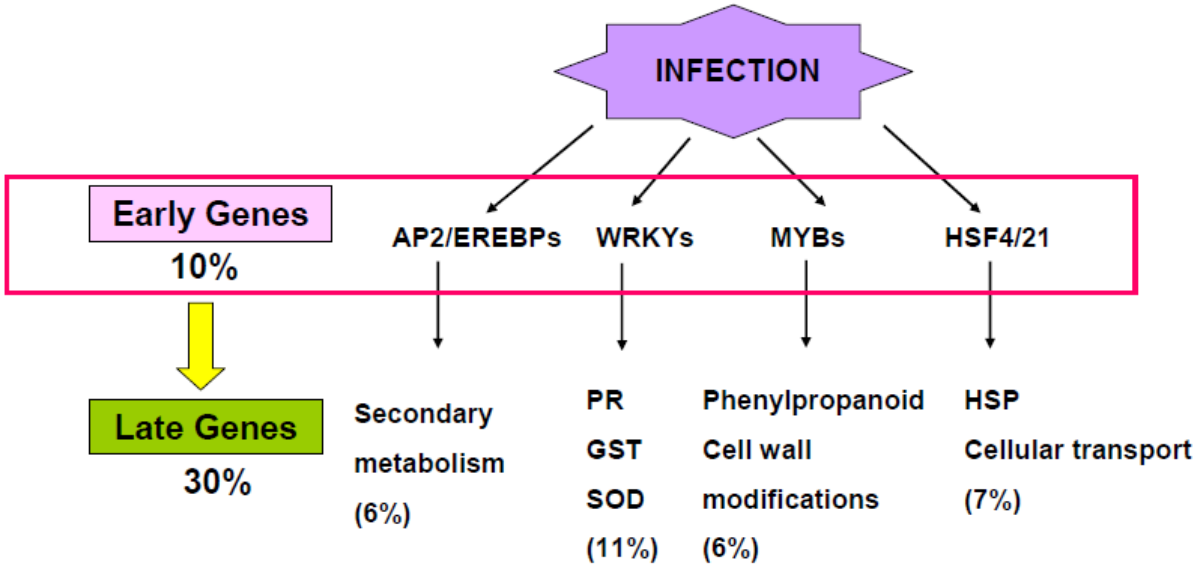


Fig. 5.1 A summary of the main categories of early infection-responsive transcription factors that activate late responsive genes during *F. verticillioides* infection in maize kernels. The percentage of genes involved in defense response is reported in brackets.

To verify if basal resistance was common to other maize genotypes and inhibited several pathogens spread after successfull infection, in the third part of the work, ten resistant and susceptible lines were infected by different mycotoxin producing fungal species. All genotypes were able to induce the expression of a defense gene upon infection with all the fungal species considered and in particular the susceptible genotype responded more intensely to pathogen infection. Furthermore all the resistant lines evaluated showed a basal defense to *F. verticillioides*.

Plant resistance to biotrophic pathogens is classically thought to be mediated through SA signaling. The involvement of SA as signal molecule in local defenses and in systemic acquired resistance have been extensively studied (Fobert and Després, 2005). Increased endogenous levels of SA and its conjugates in pathogen-inoculated plants coincide with the elevated expression of genes encoding the PR proteins and the activation of disease resistance. It is known that in plants SA can be methylated to form methyl SA. This volatile compound is biologically inactive and

considered to be an airbone signal that induces the defense responses in nondamaged organs of the same plant or in adjacent ones, being absorbed by the tissue and then converted back into free active SA by an esterase activity (Forouhar *et al*., 2005). Our hypothesis of work is that basal resistance showed by resistant genotypes before infection is linked to signal transduction mechanisms of SA. To confirm the hypothesis, biochemical events regarding SA signaling will be measured in kernels of different resistant and susceptible genotypes before and after infection by *F. verticillioides*.

Moreover, central components of many aspects of plant basal resistance are the WRKY transcriptional factors (Rients and Thierry, 2009; Rushton *et al.,* 2010). WRKY proteins mediate links between major defense signaling pathways involving MAP kinases and resistance proteins. This is an active research area that will be exploited in the future to unravel some components of the basal response mediated by WRKY.

Chapter 6

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Chapter 7

Appendix: Supplementary data.

Supplementary Table 1. Differentially expressed genes in CO441 after *Fusarium verticillioides* infection.		
ID	**Putative Annotation**	**p-value**
MZ00004694	NA	2.06E-09
MZ00025889	starch synthase DULL1 - maize {Zea mays}	2.15E-09
MZ00040688	11 kDa methionine-rich protein {Zea mays}	2.53E-09
MZ00013458	putative zinc finger protein ZmZf {Zea mays}	2.67E-09
MZ00021999	unknown protein {Oryza sativa}	3.06E-09
MZ00030905	NA	3.80E-09
MZ00051767	putative acetyl transferase {Oryza sativa}	4.06E-09
MZ00057233	unknown protein {Oryza sativa}	4.34E-09
MZ00015027	terpene synthase 5 {Zea mays}	4.44E-09
MZ00014973	Putative leucine-rich repeat transmembrane protein kinase {Oryza sativa}	4.75E-09
MZ00015063	hypothetical protein {Oryza sativa}	5.07E-09
MZ00025208	putative ABI3-interacting protein 2; CnAIP2 {Oryza sativa}	5.28E-09
MZ00021719	NA	6.03E-09
MZ00041780	zinc metalloproteinase-like {Oryza sativa}	6.74E-09
MZ00038140	10K zein precursor - {Zea mays}	8.24E-09
MZ00046894	gibberellin response modulator-like protein {Oryza sativa}	4.31E-08
MZ00023408	putative histone H2A {Oryza sativa}	6.82E-08
MZ00052479	hypothetical protein {Oryza sativa}	8.83E-08
MZ00031722	hypothetical protein similar to Arabidopsis thaliana {Oryza sativa}	9.29E-07
MZ00036117	thaumatin-like protein {Zea mays}	0.000111
MZ00035387	NA	0.000112
MZ00022990	NA	0.000116
MZ00029640	putative glycosyl hydrolase family protein {Oryza sativa}	0.000123
MZ00031041	putative aldose 1-epimerase - like protein {Oryza sativa}	0.000126
MZ00029293	hypothetical protein {Oryza sativa}	0.000135
MZ00023675	OSJNBa0085I10.2 {Oryza sativa }	0.000137
MZ00038352	contains EST AU163572(E1976) similar to ankyrin unknown protein {Oryza sativa}	0.000138

MZ00026595	unknown protein {Oryza sativa}	0.000139
MZ00054915	putative family II lipase EXL4 {Oryza sativa}	0.000143
MZ00046465	lycogenin glucosyltransferase (glycogenin)-like protein {Oryza sativa}	0.000143
MZ00055744	putative hydroxyproline-rich glycoprotein {Oryza sativa}	0.000147
MZ00018950	knotted1-like homeodomain protein liguleless4a {Zea mays}	0.000152
MZ00030566	NA	0.000160
MZ00040763	hypothetical protein BET1 - {Zea mays}	0.000165
MZ00032301	NA	0.000166
MZ00004307	putative nucleoside diphosphate kinase {Oryza sativa}	0.000197
MZ00036257	expressed protein {Oryza sativa}	0.000217
MZ00037140	glucose starvation-induced protein precursor (clone pZSS2) {Zea mays}	0.000221
MZ00005959	subtilisin/chymotrypsin inhibitor {Zea mays}	0.000245
MZ00033404	unknown protein {Oryza sativa}	0.000250
MZ00044068	adenosine diphosphate glucose pyrophosphatase {Hordeum vulgare}	0.000262
MZ00048704	NA	0.000293
MZ00042006	OSJNBa0060P14.17 {Oryza sativa}	0.000302
MZ00047925	WRKY13 {Oryza sativa}	0.000302
MZ00050372	hypothetical protein {Oryza sativa}	0.000313
MZ00043659	pathogenesis-related protein 4 {Triticum monococcum}	0.000337
MZ00016657	AT3g05970/F2O10_9 {Arabidopsis thaliana}	0.000358
MZ00055726	NA	0.000362
MZ00035180	18kD delta zein {Zea mays;}	0.000364
MZ00045598	NA	0.000399
MZ00001717	similar to an Arabidopsis putative P-type transporting ATPase {Oryza sativa}	0.000402
MZ00043033	MEG2 {Zea mays}	0.000403
MZ00032940	putative aminoacylase {Oryza sativa}	0.000406
MZ00003797	NA	0.000434
MZ00022549	putative mitochondrial intermediate peptidase {Oryza sativa}	0.000464
MZ00043891	NA	0.000473

MZ00043034	NA	0.000483
MZ00012902	NA	0.000484
MZ00034841	psaI gene {Zea mays}	0.000485
MZ00042886	cytoplasmic aldolase {Zea mays}	0.000486
MZ00021942	NA	0.000508
MZ00025221	OSJNBa0043A12.24 {Oryza sativa}	0.000554
MZ00023948	photosystem I protein-like protein {Oryza sativa}	0.000582
MZ00036738	putative diphosphate-fructose-6-phosphate 1-phosphotransferase {Oryza sativa}	0.000593
MZ00034325	OSJNBa0033G16.4 {Oryza sativa}	0.000595
MZ00026976	putative membrane protein {Oryza sativa}	0.000599
MZ00018286	respiratory burst oxidase protein B {Oryza sativa}	0.000602
MZ00055339	NA	0.000621
MZ00041442	putative snRNP core protein SMX5d {Oryza sativa}	0.000638
MZ00033113	putative adenosylmethionine-8-amino-7- oxononanoate aminotransferase {Oryza sativa}	0.000639
MZ00030326	NA	0.000678
MZ00022020	globulin-1 {Zea mays}	0.000689
MZ00041075	putative 60S ribosomal protein L18a {Oryza sativa}	0.000705
MZ00055001	dopamine beta-monooxygenase N-terminal domain-containing protein {Oryza sativa}	0.000717
MZ00014982	unknown protein {Oryza sativa}	0.000755
MZ00047199	putative male sterility 1 protein {Oryza sativa}	0.000756
MZ00055796	putative glucose-6-phosphate/phosphate- translocator precursor {Oryza sativa}	0.000801
MZ00031627	unknown protein {Oryza sativa}	0.000804
MZ00007249	expressed protein {Oryza sativa}	0.000809
MZ00044645	NA	0.000813
MZ00013957	Unknown protein {Oryza sativa}	0.000872
MZ00035363	lysine decarboxylase-like protein {Oryza sativa}	0.000874
MZ00024456	unknown protein {Oryza sativa}	0.000967
MZ00032963	NA	0.000983
MZ00043159	OSJNBb0039L24.13 {Oryza sativa)	0.000984

MZ00055677	NA	0.000989
MZ00018323	putative dex1 protein {Oryza sativa}	0.001019
MZ00006844	NA	0.001037
MZ00048726	alpha-rhamnosidase-like protein {Oryza sativa}	0.001046
MZ00014370	60S acidic ribosomal protein P2B. {Zea mays}	0.001070
MZ00056564	putative CRT/DRE binding factor 1 {Oryza sativa}	0.001079
MZ00030660	unknown protein {Oryza sativa}	0.001080
MZ00038969	NA	0.001088
MZ00027461	OJ000114_01.13 {Oryza sativa }	0.001112
MZ00003794	unknown protein {Oryza sativa }	0.001134
MZ00034385	putative beta-ketoacyl-CoA synthase {Oryza sativa }	0.001145
MZ00041377	putative succinate dehydrogenase flavoprotein alpha subunit {Oryza sativa }	0.001148
MZ00022287	zinc finger POZ domain protein-like {Oryza sativa }	0.001154
MZ00001119	NA	0.001162
MZ00031962	Alpha-amylase inhibitor 5 (SI alpha-5). {Sorghum bicolor;}	0.001173
MZ00035730	Profilin A. {Oryza sativa;}	0.001190
MZ00043074	putative ribosomal protein {Oryza sativa }	0.001191
MZ00019000	unknown protein {Oryza sativa }	0.001198
MZ00046765	hypothetical protein {Oryza sativa }	0.001201
MZ00047775	OSJNBb0059K02.13 {Oryza sativa }	0.001203
MZ00048761	myb family transcription factor-like {Oryza sativa }	0.001204
MZ00001392	putative stripe rust resistance protein Yr10 {Sorghum bicolor;}	0.001216
MZ00004700	putative metacaspase, having alternative splicing products {Oryza sativa }	0.001224
MZ00031728	NA	0.001232
MZ00018075	NA	0.001235
MZ00041327	Bowman-Birk type trypsin inhibitor (WTI). {Triticum aestivum;}	0.001236
MZ00029487	NA	0.001253
MZ00043150	putative 60S ribosomal protein L11 {Oryza sativa }	0.001253
MZ00004979	NA	0.001266

MZ00044177	unknown protein {Oryza sativa }	0.001275
MZ00007134	unknown protein {Arabidopsis thaliana}	0.001284
MZ00039815	NA	0.001311
MZ00023736	40S ribosomal protein S21. {Zea mays}	0.001316
MZ00029527	CRS1 {Zea mays}	0.001321
MZ00033356	putative membrane protein {Oryza sativa }	0.001331
MZ00044219	integral membrane protein, putative {Arabidopsis thaliana}	0.001361
MZ00001531	putative histidine amino acid transporter {Oryza sativa }	0.001366
MZ00046858	NA	0.001384
MZ00020437	O-methyltransferase ZRP4 (EC 2.1.1.-) (OMT). {Zea mays}	0.001386
MZ00043093	contains EST AU070442(S15668) unknown protein {Oryza sativa }	0.001387
MZ00056907	transcription factor-like {Oryza sativa }	0.001395
MZ00045641	NA	0.001429
MZ00042223	NA	0.001454
MZ00040247	PDI-like protein {Zea mays}	0.001467
MZ00020990	putative ATP-dependent transmembrane transporter {Oryza sativa }	0.001473
MZ00046520	putative Nt-gh3 deduced protein {Oryza sativa }	0.001494
MZ00014363	Bowman-Birk type wound induced proteinase inhibitor WIP1 precursor. {Zea mays}	0.001511
MZ00052106	hypothetical protein {Oryza sativa }	0.001518
MZ00025558	probable ubiquitin activating enzyme [imported] - {Arabidopsis thaliana}	0.001529
MZ00047985	unknown protein {Oryza sativa }	0.001570
MZ00016202	NA	0.001574
MZ00019339	OSJNBb0034I13.13 {Oryza sativa }	0.001617
MZ00055977	NA	0.001619
MZ00006051	unknown protein {Oryza sativa }	0.001629
MZ00045633	NA	0.001641
MZ00040989	NA	0.001644
MZ00031973	NA	0.001648
MZ00035263	glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) {Zea mays}	0.001657

MZ00036568	putative asparaginyl-tRNA synthetase {Oryza sativa }	0.001658
MZ00039327	metallothionein-like protein {Cynodon dactylon;}	0.001663
MZ00012649	unknown protein {Oryza sativa }	0.001672
MZ00031775	sigma factor SIG2B {Zea mays}	0.001687
MZ00033239	NA	0.001694
MZ00024153	ABC transporter homolog {Populus nigra;}	0.001701
MZ00039305	Metallothionein-like protein type 2. {Oryza sativa;}	0.001735
MZ00049620	Importin beta-like protein {Oryza sativa (indica cultivar-group);}	0.001741
MZ00015103	Similar to Arabidopsis thaliana chromosome II BAC T06D20 genomic sequence	0.001747
MZ00032902	CAF protein {Arabidopsis thaliana}	0.001748
MZ00013902	putative dormancy-asociated protein {Oryza sativa;}	0.001759
MZ00041301	basal layer antifungal peptide {Zea mays}	0.001765
MZ00025941	OJ000315_02.1 {Oryza sativa }	0.001820
MZ00036403	proline-rich protein {Zea mays}	0.001825
MZ00052045	BWF1-like protein {Oryza sativa }	0.001833
MZ00049098	NA	0.001834
MZ00032214	1-phosphatidylinositol-3-phosphate 5-kinase FAB1-like protein {Oryza sativa }	0.001834
MZ00014683	putative ribosomal protein I {Oryza sativa }	0.001865
MZ00001629	NA	0.001867
MZ00035052	pathogenesis related protein-5 - {Zea mays}	0.001877
MZ00013067	receptor-like kinase {Zea mays}	0.001881
MZ00036530	unnamed protein product {Kluyveromyces lactis NRRL Y-1140;}	0.001889
MZ00048814	putative nucleoid DNA-binding protein cnd41 {Oryza sativa }	0.001962
MZ00017059	putative polyribonucleotide nucleotidyltransferase {Oryza sativa }	0.001969
MZ00051256	pentatricopeptide (PPR) repeat-containing protein-like {Oryza sativa }	0.001977
MZ00044289	putative stromal cell-derived factor 2 precursor {Oryza sativa }	0.001979
MZ00034213	putative delta 1-pyrroline-5-carboxylate synthetase {Oryza sativa }	0.002000
MZ00021296	putative Avr9/Cf-9 rapidly elicited protein {Oryza sativa }	0.002001
MZ00037794	NA	0.002008

MZ00004709	unknown protein {Oryza sativa }	0.002018
MZ00040224	plasma membrane Ca2+-ATPase {Glycine max;}	0.002100
MZ00036685	NA	0.002110
MZ00027617	hypothetical protein {Oryza sativa }	0.002118
MZ00039719	NA	0.002125
MZ00055050	MGC80941 protein {Xenopus laevis;}	0.002135
MZ00022324	transport inhibitor response-like protein {Arabidopsis thaliana}	0.002141
MZ00055766	senescence-associated protein-like {Oryza sativa }	0.002158
MZ00022500	NA	0.002168
MZ00020845	unknown protein {Sorghum bicolor;}	0.002180
MZ00030287	NA	0.002197
MZ00041844	OSJNBa0060N03.9 {Oryza sativa }	0.002205
MZ00044478	hypothetical protein - {Zea mays} AGAMOUS-like protein {Zea mays}	0.002224
MZ00028022	putative ethanolamine kinase 1 {Oryza sativa }	0.002225
MZ00047663	NA	0.002239
MZ00044632	putative pumilio/Mpt5 family RNA-binding protein {Oryza sativa;}	0.002240
MZ00026316	unknown protein {Oryza sativa }	0.002267
MZ00021252	NA	0.002281
MZ00012680	putative CBL-interacting protein kinase {Oryza sativa }	0.002311
MZ00022394	NA	0.002321
MZ00039959	acidic ribosomal protein P2 - {Zea mays}	0.002351
MZ00031065	OSJNBb0086G13.8 {Oryza sativa }	0.002417
MZ00037227	zinc finger transcription factor ZF1 {Oryza sativa (indica cultivar-group);}	0.002445
MZ00051324	hypothetical protein {Arabidopsis thaliana}	0.002455
MZ00036825	AT5g20250/F5O24_140 {Arabidopsis thaliana}	0.002468
MZ00000984	putative Ras-GTPase activating protein SH3 domain-binding protein 2 {Oryza sativa }	0.002533
MZ00041559	NAM-related protein 1 {Zea mays}	0.002556
MZ00017614	lysophospholipase isolog, putative {Arabidopsis thaliana}	0.002565
MZ00037097	At1g72060 {Arabidopsis thaliana}	0.002575

MZ00005795	NA	0.002590
MZ00038840	NA	0.002605
MZ00048755	putative heat shock protein 40 {Oryza sativa }	0.002633
MZ00016711	NA	0.002635
MZ00019535	NA	0.002638
MZ00024884	expressed protein {Oryza sativa }	0.002665
MZ00038870	OSJNBb0004G23.1 {Oryza sativa }	0.002667
MZ00039042	NA	0.002674
MZ00039515	putative zinc finger protein {Arabidopsis thaliana}	0.002677
MZ00001280	hypothetical protein AT4g05210 {Arabidopsis thaliana}	0.002698
MZ00046462	putative amino acid permease {Oryza sativa }	0.002724
MZ00042670	NA	0.002725
MZ00038879	NA	0.002734
MZ00031363	hypothetical protein {Oryza sativa }	0.002760
MZ00007077	NA	0.002787
MZ00001664	prpol {Zea mays}	0.002796
MZ00023435	NA	0.002807
MZ00038153	hypothetical protein {Oryza sativa }	0.002822
MZ00048780	unknown protein {Oryza sativa }	0.002825
MZ00037086	NA	0.002842
MZ00041357	Similar to Caenorhabditis elegans cosmid T10C6. (Z93388) {Oryza sativa }	0.002849
MZ00037432	NA	0.002849
MZ00006865	NA	0.002853
MZ00028567	OSJNBa0020I02.8 {Oryza sativa }	0.002854
MZ00023180	putative Avr9/Cf-9 rapidly elicited protein 141 {Oryza sativa }	0.002856
MZ00043089	beta-expansin 8 {Zea mays}	0.002868
MZ00052295	NA	0.002868
MZ00016746	putative inositol-1-monophosphatase {Oryza sativa }	0.002888
MZ00005554	NA	0.002912

MZ00032845	NA	0.002936
MZ00007584	hypothetical protein T5A14.5 {Arabidopsis thaliana}	0.002939
MZ00031458	hypothetical protein T20K14_80 {Arabidopsis thaliana}	0.002967
MZ00020830	putative SNF2 domain-containing protein {Oryza sativa }	0.002968
MZ00028685	putative lecithin diacylglycerol cholesterol acyltransferase {Oryza sativa }	0.002978
MZ00032835	putative ABC1 protein {Oryza sativa }	0.002999
MZ00026218	endosperm specific protein SC3 - {Zea mays}	0.003015
MZ00005831	putative aurora-related kinase {Oryza sativa }	0.003037
MZ00023919	G-box binding protein-like {Oryza sativa }	0.003038
MZ00029826	NA	0.003052
MZ00027548	NA	0.003077
MZ00035265	NA	0.003079
MZ00036129	NA	0.003100
MZ00021449	NA	0.003108
MZ00005096	hypothetical protein {Oryza sativa } putative glycine rich protein {Oryza sativa}	0.003109
MZ00034306	putative elongation factor 1-gamma {Oryza sativa }	0.003123
MZ00040721	putative rp3 protein {Zea mays}	0.003123
MZ00028739	hydrolase-like protein {Oryza sativa }	0.003133
MZ00035258	Pyruvate decarboxylase isozyme 3 (EC 4.1.1.1) {Zea mays}	0.003141
MZ00041303	zinc finger and C2 domain protein-like {Oryza sativa }	0.003158
MZ00015836	LEM3-like {Oryza sativa }	0.003167
MZ00005198	NA	0.003201
MZ00039967	NA	0.003203
MZ00005623	putative PDR-type ABC transporter 9 {Oryza sativa }	0.003221
MZ00030272	putative actin {Arabidopsis thaliana}	0.003239
MZ00034239	centromere protein-like {Oryza sativa }	0.003244
MZ00037898	copia protein {Zea mays}	0.003245
MZ00000930	hypothetical protein {Oryza sativa }	0.003260
MZ00036636	probable trypsin inhibitor - {Zea mays}	0.003276

MZ00055342	NA	0.003278
MZ00018988	putative acetylornithine aminotransferase {Oryza sativa }	0.003278
MZ00020211	hypothetical protein {Arabidopsis thaliana}	0.003283
MZ00022327	NA	0.003304
MZ00018284	subtilisin-like proteinase (EC 3.4.21.-) - {Arabidopsis thaliana}	0.003304
MZ00023228	putative cinnamoyl-CoA reductase {Oryza sativa }	0.003315
MZ00004832	unknown protein {Oryza sativa }	0.003327
MZ00033277	NA	0.003356
MZ00046519	putative serine/threonine-specific protein kinase {Oryza sativa }	0.003373
MZ00035495	hypothetical protein Y39B6B {Caenorhabditis elegans;}	0.003394
MZ00000704	putative LRR receptor-like kinase 2 {Oryza sativa }	0.003401
MZ00055874	NA	0.003432
MZ00020134	putative receptor Mediated Endocytosis RME-1 {Oryza sativa }	0.003439
MZ00030430	patatin-like protein {Oryza sativa }	0.003461
MZ00028500	putative RNA polymerase I subunit {Oryza sativa }	0.003493
MZ00020565	putative U5 snRNP-specific 40 kDa protein {Oryza sativa }	0.003500
MZ00004521	NA	0.003522
MZ00039450	CSLC2 {Oryza sativa;}	0.003540
MZ00052159	unknown protein {Oryza sativa }	0.003570
MZ00017490	unknown protein {Oryza sativa }	0.003576
MZ00043724	unknown {Arabidopsis thaliana}	0.003584
MZ00046572	NA	0.003591
MZ00020736	expressed protein {Arabidopsis thaliana}	0.003592
MZ00040208	NA	0.003595
MZ00021547	NA	0.003613
MZ00004326	arginine/serine-rich splicing factor 1 {Zea mays}	0.003613
MZ00037925	putative ribosomal protein L17 {Oryza sativa }	0.003617
MZ00020970	putative bHLH transcription factor (AC006418) {Oryza sativa}	0.003650
MZ00027755	putative DNA-binding protein {Oryza sativa }	0.003656

MZ00018709	putative transposon protein {Oryza sativa }	0.003656
MZ00026715	NA	0.003659
MZ00056415	putative DNA damage repair protein {Oryza sativa }	0.003678
MZ00052373	NA	0.003680
MZ00014498	prohibitin {Zea mays}	0.003704
MZ00037526	putative gag-pol polyprotein {Zea mays}	0.003721
MZ00028849	glucan endo-1,3-beta-D-glucosidase (EC 3.2.1.39){Nicotiana tabacum;}	0.003723
MZ00054831	PWWP domain protein-like {Oryza sativa }	0.003730
MZ00042024	Similar to putative argonaute protein (AC005623) {Oryza sativa }	0.003734
MZ00021975	unknown protein {Oryza sativa }	0.003742
MZ00020012	NA	0.003822
MZ00033367	hypothetical protein {Oryza sativa }	0.003825
MZ00004456	unknown protein {Oryza sativa }	0.003833
MZ00016213	unknown protein {Oryza sativa }	0.003842
MZ00020049	hypothetical protein F28A23.150 - {Arabidopsis thaliana}	0.003862
MZ00016489	unknown protein {Oryza sativa }	0.003863
MZ00033535	NA	0.003892
MZ00007041	F10B6.25 {Arabidopsis thaliana}	0.003896
MZ00042111	unknown protein {Oryza sativa }	0.003898
MZ00023205	putative 5-3 exoribonuclease {Oryza sativa }	0.003909
MZ00032280	NA	0.003924
MZ00025158	Sec13p {Oryza sativa }	0.003926
MZ00030310	similar to Arabidopsis thaliana chromosome1,At1g34320	0.003928
MZ00047746	NA	0.003935
MZ00018488	response regulator 7 {Zea mays}	0.003964
MZ00036454	probable auxin transport protein - rice {Oryza sativa;}	0.003985
MZ00043183	NA	0.003990
MZ00014343	unknown protein {Oryza sativa }	0.003990
MZ00029323	lipase-like protein {Arabidopsis thaliana}	0.003998

MZ00022650	putative phosphatidylinositol 4-kinase {Oryza sativa;}	0.004000
MZ00035414	synthase proteolipid subunit,ATP. {Spinacia sp.;} ATPase III. {Oryza sativa;}	0.004031
MZ00031362	unknown protein {Oryza sativa }	0.004040
MZ00015318	Thiazole biosynthetic enzyme 1-1, chloroplast precursor. {Zea mays}	0.004045
MZ00013780	protein phosphatase 2C-like {Oryza sativa }	0.004052
MZ00007427	proline-rich protein 13 {Rattus norvegicus}	0.004071
MZ00048560	nodulin-like protein {Oryza sativa }	0.004081
MZ00049575	hydroxyproline-rich glycoprotein DZ-HRGP {Volvox carteri f. nagariensis;}	0.004092
MZ00026305	OSJNBa0039K24.2 {Oryza sativa }	0.004093
MZ00042423	NA	0.004128
MZ00004702	NA	0.004129
MZ00013509	putative inorganic pyrophosphatase {Oryza sativa }	0.004131
MZ00032113	NA	0.004139
MZ00015621	hypothetical protein F24J5.11 [imported] - {Arabidopsis thaliana}	0.004160
MZ00040783	yabby10 protein {Zea mays}	0.004174
MZ00056967	NA	0.004190
MZ00032744	NA	0.004214
MZ00044311	NA	0.004217
MZ00040574	pol protein homolog - maize retrotransposon Opie-2 {Zea mays}	0.004228
MZ00021057	OSJNBb0020J19.18 {Oryza sativa }	0.004250
MZ00044709	unknown protein {Oryza sativa }	0.004251
MZ00029562	hypothetical protein {Oryza sativa }	0.004260
MZ00048700	hypothetical protein similar to Arabidopsis thaliana chromosome 1,At1g71720	0.004267
MZ00030107	harpin-induced family protein-like {Oryza sativa }	0.004268
MZ00044487	Anthocyanin regulatory Lc protein. {Zea mays}	0.004271
MZ00005231	ribosomal protein L15 {Oryza sativa }	0.004287
MZ00013647	NA	0.004297
MZ00047076	putative DnaJ domain containg protein {Oryza sativa }	0.004307
MZ00029394	OSJNBa0064G10.18 {Oryza sativa }	0.004334

MZ00037371	3,4-dihydroxy-2-butanone kinase {Oryza sativa }	0.004353
MZ00037815	NA	0.004387
MZ00026964	annexin max4 {Oryzias latipes;}	0.004388
MZ00044102	Hypothetical protein At3g06530. {Arabidopsis thaliana}	0.004444
MZ00025854	unknown protein {Oryza sativa }	0.004456
MZ00014792	putative alpha-coat protein {Oryza sativa }	0.004466
MZ00036023	putative protein kinase 5 {Oryza sativa }	0.004468
MZ00024415	putative leucine-rich repeat transmembrane protein kinase {Arabidopsis thaliana}	0.004482
MZ00035844	unnamed protein product; no similarity, hypothetical start {Kluyveromyces lactis}	0.004506
MZ00023006	putative endoribonuclease E {Oryza sativa }	0.004509
MZ00013117	hypothetical protein similar to Arabidopsis thaliana chromosome 1, At1g15490	0.004510
MZ00035508	putative F-box protein {Oryza sativa }	0.004517
MZ00001591	OSJNBa0033G05.7 {Oryza sativa }	0.004573
MZ00004791	Unknown {Vibrio vulnificus CMCP6;}	0.004582
MZ00039779	hypothetical protein {Oryza sativa }	0.004588
MZ00047133	hypothetical protein similar to Arabidopsis thaliana chromosome 4, At4g18460	0.004589
MZ00013464	putative 60S ribosomal protein L31 {Oryza sativa }	0.004600
MZ00027979	putative ROP family GTPase ROP7 {Zea mays}	0.004604
MZ00030339	unknown protein {Oryza sativa }	0.004631
MZ00048983	similar to Arabidopsis thaliana chromosome 1, unknown protein {Oryza sativa }	0.004661
MZ00044510	NA	0.004669
MZ00014759	unknown protein {Oryza sativa }	0.004706
MZ00014804	NA	0.004707
MZ00057401	NA	0.004717
MZ00041329	pyruvate dehydrogenase E1 alpha subunit {Zea mays}	0.004726
MZ00013605	probable isoamylase (EC 3.2.1.68) su1 {Zea mays}	0.004732
MZ00025651	Superoxide dismutase [Cu-Zn], chloroplast precursor (EC 1.15.1.1). {Oryza sativa;}	0.004734
MZ00021786	OSJNBb0108J11.6 {Oryza sativa }	0.004742
MZ00003536	auxin response factor 2 {Oryza sativa;}	0.004747

MZ00035835	unnamed protein product {Kluyveromyces lactis NRRL Y-1140;}	0.004760
MZ00043323	NA	0.004761
MZ00014420	putative ATP synthase gamma chain 1, chloroplast (H(+)-transporting two-sector ATPase	0.004789
MZ00039451	NA	0.004801
MZ00016021	hypothetical protein [imported] - {Arabidopsis thaliana}	0.004819
MZ00038392	putative UDP-glucose dehydrogenase {Oryza sativa }	0.004832
MZ00041399	sterol 24-C-methyltransferase (EC 2.1.1.41) ESMT1, endosperm - {Zea mays}	0.004841
MZ00025302	contains EST AU094821(E31815) unknown protein {Oryza sativa }	0.004844
MZ00031809	xyloglucan endo-1,4-beta-D-glucanase (EC 3.2.1.-) - {Hordeum vulgare}	0.004864
MZ00001501	Lsp1beta {Drosophila buzzatii;}	0.004864
MZ00026470	putative trehalose-6-phosphate phosphatase {Oryza sativa }	0.004868
MZ00040929	translation elongation factor eEF-1 beta - rice {Oryza sativa;}	0.004891
MZ00019311	putative nicastrin {Oryza sativa }	0.004902
MZ00040223	NA	0.004911
MZ00041030	UDP-glucose pyrophosphorylase {Bambusa oldhamii;}	0.004925
MZ00056811	putative Serine/threonine phosphatases {Oryza sativa }	0.004929
MZ00029071	similar to Arabidopsis thaliana transport inhibitor response 1 (T48087) {Oryza sativa}	0.004929
MZ00044657	R2R3MYB-domain protein {Zea mays}	0.004949
MZ00022301	KNOX family class 2 homeodomain protein {Oryza sativa }	0.004955
MZ00013222	NA	0.004974
MZ00021912	S-ribonuclease binding protein SBP1-like {Oryza sativa }	0.004987
MZ00031797	GCN5-related N-acetyltransferase-like {Oryza sativa }	0.005036
MZ00030651	putative cyclophilin {Oryza sativa }	0.005155
MZ00039007	lipoxygenase {Zea mays}	0.005157
MZ00031393	similar to Arabidopsis thaliana chromosome5,At5g58560 unknown protein {Oryza sativa }	0.005160
MZ00041770	serine-tRNA ligase (EC 6.1.1.11) - {Zea mays} seryl-tRNA synthetase {Zea mays}	0.005186
MZ00013941	Profilin A. {Oryza sativa}	0.005234
MZ00007600	OSJNBb0017I01.21 {Oryza sativa }	0.005242
MZ00032573	similar to Arabidopsis thaliana chromosome 5, At5g44680 unknown protein {Oryza sativa }	0.005247

MZ00034959	Ribosomal protein S7 {Hordeum vulgare subsp. vulgare;}	0.005258
MZ00042448	putative latex protein allergen {Oryza sativa }	0.005264
MZ00044664	rust resistance Rp1-D-like protein {Zea mays}	0.005271
MZ00042266	NA	0.005291
MZ00055648	disease relative signal 1 {Oryza sativa } putative protein kinase {Oryza sativa}	0.005294
MZ00051892	putative inositol 1,4,5-trisphosphate 5-phosphatase {Oryza sativa }	0.005323
MZ00049661	DNA-binding protein-like {Oryza sativa }	0.005334
MZ00056881	putative aspartate kinase {Oryza sativa }	0.005336
MZ00032216	hypothetical protein {Oryza sativa }	0.005342
MZ00039524	BETL2 protein {Zea mays}	0.005366
MZ00015167	putative asparaginyl-tRNA synthetase, chloroplast/mitochondrial precursor {Oryza sativa }	0.005370
MZ00017923	putative insulin degrading enzyme {Oryza sativa }	0.005376
MZ00030389	contains EST C26290(C12038) unknown protein {Oryza sativa }	0.005381
MZ00004306	NA	0.005381
MZ00019361	NA	0.005395
MZ00005088	NA	0.005423
MZ00001559	NA	0.005442
MZ00046842	putative heparanase {Hordeum vulgare subsp. vulgare;}	0.005452
MZ00028737	similar to Arabidopsis thaliana chromosome 1, F14G9.16 unknown protein {Oryza sativa }	0.005476
MZ00048588	similar to transcription factor SCARECROW {Oryza sativa }	0.005485
MZ00028498	putative peroxidase precursor {Oryza sativa }	0.005506
MZ00004100	hypothetical protein At2g28530 [imported] - {Arabidopsis thaliana}	0.005506
MZ00039184	beta-glucosidase, root meristem (EC 3.2.1.-) precursor - {Zea mays}	0.005509
MZ00030619	putative serine/threonine protein kinase {Ipomoea batatas;}	0.005522
MZ00024180	putative plastid protein {Oryza sativa }	0.005543
MZ00004761	mitochondrial transcription termination factor-like {Oryza sativa }	0.005567
MZ00027689	hypothetical protein - {Arabidopsis thaliana}	0.005584
MZ00042422	OSJNBb0004G23.3 {Oryza sativa } OSJNBa0094O15.17 {Oryza sativa }	0.005585
MZ00024336	nucleic acid binding protein - rice {Oryza sativa;}	0.005596

MZ00017165	DNAJ protein-like {Arabidopsis thaliana}	0.005597
MZ00028768	unknown protein {Oryza sativa }	0.005600
MZ00022825	putative plastidic ATP/ADP-transporter {Oryza sativa }	0.005614
MZ00050570	putative protein transport SEC23-related protein {Oryza sativa }	0.005618
MZ00039447	NA	0.005621
MZ00041606	putative polyketide synthase {Oryza sativa }	0.005631
MZ00030391	guanine nucleotide-exchange protein GEP2 {Oryza sativa;}	0.005655
MZ00042047	putative senescence-associated protein 12 {Arabidopsis thaliana}	0.005659
MZ00017969	putative phenylalanyl-tRNA synthetase alpha chain {Oryza sativa }	0.005688
MZ00031685	10-formyltetrahydrofolate synthetase [imported] - {Arabidopsis thaliana}	0.005690
MZ00029146	unknown protein {Oryza sativa }	0.005694
MZ00052117	NA	0.005726
MZ00022188	probable protein kinase [imported] - Arabidopsis thaliana {Arabidopsis thaliana}	0.005745
MZ00005620	NA	0.005748
MZ00005382	putative iron-sulfur cluster-binding protein {Oryza sativa }	0.005750
MZ00012911	OSJNBa0017B10.2 {Oryza sativa }	0.005758
MZ00032551	putative elicitor response protein {Oryza sativa }	0.005775
MZ00004703	AT5g39670/MIJ24_140 {Arabidopsis thaliana}	0.005788
MZ00027189	calcium-dependent protein kinase (EC 2.7.1.-) 9 - {Zea mays}	0.005790
MZ00051211	putative transcriptional regulator {Oryza sativa }	0.005791
MZ00034862	Chloroplast 50S ribosomal protein L22. {Zea mays}	0.005799
MZ00017677	putative ABC(ATP binding cassette)1 protein {Oryza sativa }	0.005800
MZ00022147	NA	0.005827
MZ00007402	Enolase 1 (EC 4.2.1.11) (2-phosphoglycerate dehydratase 1) {Zea mays}	0.005832
MZ00017221	kinesin heavy chain {Zea mays}	0.005833
MZ00036176	pathogenesis-related protein-like protein {Oryza sativa}	0.005894
MZ00036062	NA	0.005920
MZ00020893	NA	0.005924
MZ00043097	NA	0.005939

MZ00001588	NA	0.005950
MZ00016088	unknown protein {Oryza sativa }	0.005952
MZ00041456	pyruvate dehydrogenase E1 beta subunit isoform 2 {Zea mays}	0.005957
MZ00027517	putative formamidopyrimidine-DNA glycosylase 1 {Oryza sativa }	0.005961
MZ00019803	unknown protein {Oryza sativa }	0.005979
MZ00042722	putative phospholipase A2 {Oryza sativa }	0.006013
MZ00037568	NA	0.006032
MZ00055258	similar to Arabidopsis thaliana chromosome 1, unknown protein {Oryza sativa }	0.006057
MZ00013832	myo-inositol 1-phosphate synthase {Zea mays}	0.006074
MZ00034931	photosystem II type II chlorophyll a/b binding protein {Sorghum bicolor}	0.006083
MZ00021890	protein kinase 2 {Nicotiana tabacum;}	0.006098
MZ00031293	NA	0.006104
MZ00038776	NA	0.006117
MZ00018161	Similar to human dimethylaniline monooxygenase (AC002376) {Oryza sativa }	0.006156
MZ00031990	NA	0.006161
MZ00018318	NA	0.006162
MZ00025742	putative pectin acetylesterase {Oryza sativa }	0.006172
MZ00023554	Heat shock protein 82. {Oryza sativa;}	0.006179
MZ00033186	putative sodium-dicarboxylate cotransporter {Oryza sativa }	0.006195
MZ00035643	maize EST AI621709	0.006202
MZ00004393	hypothetical protein {Oryza sativa }	0.006208
MZ00028788	P-type ATPase {Hordeum vulgare;}	0.006215
MZ00018304	hypothetical protein {Oryza sativa }	0.006216
MZ00026741	signal recognition particle 54 kDa subunit precursor {Arabidopsis thaliana}	0.006224
MZ00039130	Alanine aminotransferase 2 (EC 2.6.1.2) {Panicum miliaceum}	0.006225
MZ00044555	putative phosphoribosyl pyrophosphate synthase {Oryza sativa }	0.006228
MZ00025636	putative small ribonucleoprotein {Oryza sativa }	0.006229
MZ00033066	NA	0.006242
MZ00014533	NA	0.006284

MZ00040071	ribosomal protein L8, cytosolic - {Arabidopsis thaliana}	0.006288
MZ00024420	CIPK-like protein {Oryza sativa }	0.006299
MZ00017639	putative cyclophilin (70.8 kD) (cyp-15) {Oryza sativa }	0.006312
MZ00046457	unknown {Oryza sativa }	0.006321
MZ00004465	salicylic acid carboxyl methyltransferase 1 {Oryza sativa}	0.006325
MZ00054984	putative DNA-binding protein {Oryza sativa }	0.006358
MZ00033043	NA	0.006368
MZ00024991	unnamed protein product; Similar to DNA gyrase subunit B. (P05652) {Oryza sativa }	0.006368
MZ00041999	hypothetical protein {Oryza sativa }	0.006384
MZ00022384	NA	0.006390
MZ00018983	hypothetical protein similar to Oryza sativa chromosome1,B1088D01.3 {Oryza sativa }	0.006406
MZ00004939	hypothetical protein {Oryza sativa }	0.006426
MZ00014956	2-isopropylmalate synthase B (EC 2.3.3.13) {Lycopersicon pennellii}	0.006436
MZ00035921	putative histidine kinase {Oryza sativa }	0.006456
MZ00048232	putative pectinesterase {Oryza sativa }	0.006467
MZ00013437	60S ribosomal protein L17 {Zea mays}	0.006499
MZ00032110	NA	0.006524
MZ00021504	hypothetical protein {Oryza sativa }	0.006549
MZ00039731	Histone H2A. {Zea mays} ^|^PIR|T02076|T02076 histone H2A - {Zea mays}	0.006554
MZ00016161	putative kinase {Oryza sativa }	0.006587
MZ00035448	small zinc finger-like protein {Oryza sativa }	0.006588
MZ00020280	unnamed protein product; Similar to putative argonaute protein;	0.006599
MZ00014615	high-glucose-regulated protein 8-like {Oryza sativa }	0.006601
MZ00031016	putative stress enhanced protein {Oryza sativa }	0.006607
MZ00020969	kelch repeat-containing F-box family protein-like {Oryza sativa }	0.006611
MZ00005354	putative polyprotein {Oryza sativa }	0.006615
MZ00040194	putative mitotic checkpoint protein, 5'-partial {Oryza sativa }	0.006623
MZ00018320	putative glycine-rich protein {Oryza sativa }	0.006625
MZ00022323	RAV-like B3 domain DNA binding protein-like {Oryza sativa }	0.006626

MZ00049734	putative callose synthase 1 catalytic subunit {Oryza sativa }	0.006646
MZ00023272	unknown protein {Arabidopsis thaliana}	0.006664
MZ00021166	OSJNBb0002J11.6 {Oryza sativa }	0.006670
MZ00038052	putative prpol {Zea mays}	0.006671
MZ00016428	centromeric histone H3-like protein {Zea mays}	0.006703
MZ00031965	putative DNA repair protein XRCC1 {Arabidopsis thaliana}	0.006718
MZ00027100	contains EST AU101554(E61607) hypothetical protein {Oryza sativa }	0.006728
MZ00019753	beta3-glucuronyltransferase {Triticum aestivum;}	0.006745
MZ00038689	NA	0.006759
MZ00030571	NA	0.006769
MZ00035541	putative protein kinase {Oryza sativa }	0.006774
MZ00029925	NA	0.006783
MZ00017491	unknown protein {Oryza sativa }	0.006802
MZ00018719	hypothetical protein {Ipomoea trifida;}	0.006829
MZ00043212	NA	0.006834
MZ00032267	putative DNA helicase {Oryza sativa }	0.006835
MZ00029772	OSJNBa0028I23.20 {Oryza sativa }	0.006843
MZ00028659	OJ000315_02.17 {Oryza sativa }	0.006846
MZ00037612	NA	0.006872
MZ00024306	Probable vacuolar ATP synthase subunit F {Arabidopsis thaliana}	0.006876
MZ00051272	NA	0.006880
MZ00022329	hypothetical protein {Oryza sativa }	0.006882
MZ00049753	putative AP2-domain transcriptional regulator {Oryza sativa }	0.006919
MZ00020518	putative zinc finger POZ domain protein {Oryza sativa }	0.006934
MZ00018538	OSJNBb0004A17.11 {Oryza sativa }	0.006971
MZ00048480	Probable 3-beta-hydroxysteroid-delta(8),delta(7)-isomerase(EC 5.3.3.5) {Oryza sativa;}	0.006972
MZ00021374	unknown protein {Oryza sativa }	0.006974
MZ00015966	OSJNBa0070O11.5 {Oryza sativa }	0.007007
MZ00022916	putative polygalacturonase PG2 {Oryza sativa }	0.007009

MZ00020493	NA	0.007010
MZ00034432	NA	0.007026
MZ00042852	NA	0.007028
MZ00042900	Metallothionein-like protein type 2. {Oryza sativa;}	0.007029
MZ00025693	putative protein tyrosine-serine-threonine kinase {Oryza sativa }	0.007056
MZ00005971	H+-exporting ATPase (EC 3.6.3.6) - {Zea mays}	0.007072
MZ00028816	unknown {Triticum aestivum;}	0.007083
MZ00025241	unknown protein {Oryza sativa }	0.007093
MZ00022414	early nodulin 75 precursor-like protein {Oryza sativa }	0.007096
MZ00039917	NA	0.007113
MZ00027363	putative latex-abundant protein {Oryza sativa }	0.007124
MZ00031356	unknown protein {Oryza sativa }	0.007131
MZ00012693	leaf senescence protein-like {Oryza sativa }	0.007207
MZ00029322	Hox12 {Oryza sativa (indica cultivar-group);}	0.007208
MZ00042369	unknown protein {Arabidopsis thaliana}	0.007210
MZ00031550	GTPase activator protein-like {Oryza sativa }	0.007223
MZ00016475	Putative FH protein interacting protein FIP1 {Oryza sativa }	0.007232
MZ00055447	similar to Arabidopsis thaliana protein T27E11.110 unknown protein {Oryza sativa }	0.007233
MZ00029092	putative 6-4 photolyase (UVR3) {Oryza sativa }	0.007236
MZ00026254	ubiquitin-specific protease 14 (UBP14), putative {Arabidopsis thaliana}	0.007249
MZ00032122	putative ubiquinone/menaquinone biosynthesis methyltransferase {Arabidopsis thaliana}	0.007255
MZ00013719	putative glycine and proline-rich protein {Sporobolus stapfianus;}	0.007258
MZ00040883	zein Zd1, 19K - {Zea mays}	0.007263
MZ00030048	hypothetical protein T12P18.17 [imported] - {Arabidopsis thaliana}	0.007265
MZ00018512	At1g03250/F15K9_13 {Arabidopsis thaliana}	0.007310
MZ00028904	myb-related gene Zm38. {Zea mays}	0.007315
MZ00021290	embryo-specific protein {Oryza sativa (indica cultivar-group);}	0.007344
MZ00013823	cysteine protease {Zea mays}	0.007345
MZ00021475	NA	0.007372

MZ00036030	branched silkless1 {Zea mays}	0.007395
MZ00021666	AT4g12830/T20K18_180 {Arabidopsis thaliana}	0.007400
MZ00052366	Cysteine proteinase 1 precursor (EC 3.4.22.-). {Zea mays}	0.007411
MZ00016860	putative Cnot10 protein {Oryza sativa }	0.007425
MZ00046926	abscisic acid- and stress-induced protein - rice {Oryza sativa;}	0.007433
MZ00021201	NA	0.007434
MZ00027571	unknown protein {Oryza sativa }	0.007487
MZ00013813	contains ESTs C73924(E21102),C99694(E21102) unknown protein {Oryza sativa }	0.007490
MZ00024928	type 1 membrane protein -like {Oryza sativa }	0.007504
MZ00017528	unknown protein {Oryza sativa }	0.007525
MZ00042589	putative 9-cis-epoxycarotenoid dioxygenase {Oryza sativa }	0.007531
MZ00030008	hypothetical protein F20D22.11 - {Arabidopsis thaliana}	0.007552
MZ00041790	putative monodehydroascorbate reductase {Oryza sativa }	0.007579
MZ00037790	gag-pol {Zea mays}	0.007583
MZ00034300	chitinase (EC 3.2.1.14) PRm 3 - maize {Zea mays}	0.007589
MZ00027764	hypothetical protein {Oryza sativa }	0.007607
MZ00016806	NA	0.007613
MZ00036847	Ac1147 {Rattus norvegicus;}	0.007624
MZ00030529	60S large subunit ribosomal protein	0.007629
MZ00049030	putative NADP-dependent oxidoreductase {Arabidopsis thaliana}	0.007635
MZ00038296	unknown protein {Arabidopsis thaliana} AT3g54190/F24B22_150 {Arabidopsis thaliana}	0.007650
MZ00017184	DNA-binding protein-like - {Arabidopsis thaliana}	0.007669
MZ00044184	OSJNBb0072N21.7 {Oryza sativa }	0.007677
MZ00031132	NA	0.007678
MZ00038706	NA	0.007692
MZ00021724	NA	0.007694
MZ00040710	cytochrome P450 monooxygenase CYP72A26 {Zea mays}	0.007696
MZ00001645	unknown protein {Oryza sativa }	0.007714
MZ00037411	copalyl diphosphate synthase like-protein {Zea mays}	0.007716

MZ00044736	putative membrane import protein {Arabidopsis thaliana}	0.007719
MZ00034963	NA	0.007757
MZ00035637	NA	0.007766
MZ00021798	putative leishmanolysin-like protein {Oryza sativa }	0.007790
MZ00027572	unknown protein {Oryza sativa }	0.007817
MZ00016272	WRKY9 {Oryza sativa }	0.007818
MZ00051908	putative rac GTPase activating protein; 62102-60058 {Arabidopsis thaliana}	0.007824
MZ00044230	putative alpha-hydroxynitrile lyase {Arabidopsis thaliana}	0.007840
MZ00005224	NA	0.007854
MZ00032609	Hypothetical protein {Oryza sativa }	0.007872
MZ00007338	putative serine protease {Oryza sativa }	0.007883
MZ00033321	putative heparanase {Hordeum vulgare subsp. vulgare;}	0.007892
MZ00001307	NA	0.007910
MZ00012953	unknown protein {Oryza sativa }	0.007957
MZ00038152	putative sorbitol dehydrogenase {Oryza sativa }	0.007967
MZ00030236	plastid division protein precursor {Oryza sativa }	0.007969
MZ00036247	NA	0.008013
MZ00023590	NA	0.008020
MZ00056842	OSJNBa0089K21.9 {Oryza sativa }	0.008046
MZ00005515	O-methyltransferase ZRP4 (EC 2.1.1.-) (OMT). {Zea mays}	0.008052
MZ00021153	NA	0.008056
MZ00036315	Photosystem I reaction centre subunit N, chloroplast precursor {Hordeum vulgare}	0.008076
MZ00028485	putative dynamin homolog {Oryza sativa }	0.008087
MZ00014641	fiber protein-like {Oryza sativa }	0.008108
MZ00022186	OSJNBa0040D17.11 {Oryza sativa }	0.008112
MZ00018936	OSJNBa0033G16.4 {Oryza sativa }	0.008113
MZ00048534	long-chain acyl-CoA synthetase {Arabidopsis thaliana}	0.008126
MZ00051341	putative receptor-like protein kinase {Oryza sativa }	0.008130
MZ00057445	pentatricopeptide repeat-containing protein -like protein {Oryza sativa }	0.008138

MZ00005405	farnesyl-pyrophosphate synthetase - rice {Oryza sativa;}	0.008144
MZ00027251	putative cytochrome P450 {Oryza sativa }	0.008151
MZ00038512	putative late embryogenesis abundant protein {Oryza sativa }	0.008164
MZ00037053	putative r40c1 protein {Oryza sativa }	0.008183
MZ00034258	serine threonine kinase {Zea mays}	0.008193
MZ00014234	putative small GTP-binding protein Bsar1a {Oryza sativa }	0.008201
MZ00021736	putative ankyrin {Oryza sativa }	0.008214
MZ00007597	Tubulin alpha-2 chain (Alpha-2 tubulin). {Zea mays}	0.008227
MZ00030952	unknown protein {Oryza sativa }	0.008241
MZ00036848	putative UNC50 {Oryza sativa }	0.008247
MZ00042322	putative peptide transport protein {Oryza sativa }	0.008263
MZ00013197	unknown {Arabidopsis thaliana}	0.008266
MZ00033633	NA	0.008269
MZ00023208	putative immunophilin {Oryza sativa }	0.008284
MZ00014605	putative polyketide synthase {Oryza sativa }	0.008285
MZ00029074	plant viral-response family protein-like {Oryza sativa }	0.008307
MZ00001709	putative WRKY transcription factor {Oryza sativa }	0.008316
MZ00016848	NA	0.008317
MZ00004049	contains eukaryotic protein kinase domain {Oryza sativa}	0.008322
MZ00023928	putative coatomer protein complex, subunit beta 2 (beta prime) {Oryza sativa }	0.008329
MZ00001482	NA	0.008358
MZ00027003	putative small nuclear ribonucleoprotein U1A {Oryza sativa;}	0.008361
MZ00026486	OSJNBa0053K19.7 {Oryza sativa }	0.008364
MZ00042068	unknown protein {Oryza sativa }	0.008369
MZ00023732	Guanine nucleotide-binding protein beta subunit {Oryza sativa}	0.008374
MZ00037777	gag-pol {Zea mays}	0.008374
MZ00048228	putative receptor protein kinase {Oryza sativa }	0.008412
MZ00013547	thaumatin-like protein. {Zea mays}	0.008417
MZ00029524	unknown protein {Oryza sativa }	0.008418

MZ00014023	40S ribosomal protein S30-like {Oryza sativa }	0.008422
MZ00038155	NA	0.008435
MZ00025932	thioredoxin h-like protein {Zea mays}	0.008440
MZ00001537	putative calcium-dependent protein kinase {Oryza sativa }	0.008449
MZ00020537	putative regulatory protein {Oryza sativa }	0.008462
MZ00034985	NA	0.008464
MZ00026248	putative adenine phosphoribosyltransferase form 2 {Oryza sativa }	0.008510
MZ00035888	histidine kinase 2 {Zea mays}	0.008523
MZ00042059	putative thioredoxin {Oryza sativa }	0.008537
MZ00001010	putative karyopherin-beta 3 variant {Oryza sativa }	0.008542
MZ00022581	homeotic protein HOX1B - maize {Zea mays}	0.008552
MZ00039157	NA	0.008597
MZ00042170	hypothetical protein {Oryza sativa }	0.008625
MZ00029484	NA	0.008637
MZ00037340	OSJNBa0028I23.16 {Oryza sativa }	0.008638
MZ00046515	putative glucosyl transferase {Sorghum bicolor;}	0.008639
MZ00043079	putative nucleic acid binding protein {Oryza sativa }	0.008661
MZ00016232	unknown protein {Oryza sativa }	0.008680
MZ00012933	NA	0.008681
MZ00005942	NA	0.008695
MZ00027669	ESTs gb|T45673,gb|N37512 come from this gene. {Arabidopsis thaliana}	0.008705
MZ00055903	putative drought-induced protein {Oryza sativa }	0.008742
MZ00037666	NA	0.008759
MZ00026482	unnamed protein product {Arabidopsis thaliana}	0.008794
MZ00043637	splicing coactivator subunit SRm300-like protein {Oryza sativa }	0.008798
MZ00014381	NA	0.008817
MZ00004904	NA	0.008820
MZ00048499	putative Myb-like DNA-binding protein {Oryza sativa }	0.008825
MZ00007133	NA	0.008837

MZ00021495	carboxyl-terminal peptidase-like {Oryza sativa }	0.008846
MZ00021181	hypothetical protein [imported] - {Arabidopsis thaliana}	0.008854
MZ00033172	MAP kinase homolog {Oryza sativa;}	0.008864
MZ00023639	hypothetical protein T14D3.120 - {Arabidopsis thaliana}	0.008882
MZ00036694	Metallothionein-like protein type 2. {Oryza sativa;}	0.008888
MZ00026051	similar to Arabidopsis thaliana chromosome 5	0.008893
MZ00033391	putative Ser/Thr protein kinase {Oryza sativa }	0.008898
MZ00018321	AP2 domain transcription factor EREBP {Oryza sativa }	0.008903
MZ00035530	putative WD-40 repeat protein {Oryza sativa }	0.008906
MZ00030735	hypothetical protein {Oryza sativa }	0.008940
MZ00024925	putative pre-mRNA splicing factor {Oryza sativa }	0.008948
MZ00023120	putative calcium-dependent protein kinase {Oryza sativa }	0.008950
MZ00017258	unknown protein {Oryza sativa }	0.008961
MZ00022597	LEC14B like-protein {Oryza sativa }	0.008978
MZ00057005	putative 60S ribosomal protein L28 {Oryza sativa }	0.008996
MZ00036046	putative lipase {Oryza sativa }	0.009001
MZ00023116	NA	0.009005
MZ00005260	NA	0.009023
MZ00019297	unknown protein {Oryza sativa }	0.009031
MZ00021247	putative protein phosphatase 2A B'kappa subunit {Oryza sativa }	0.009042
MZ00027035	unknown protein {Oryza sativa }	0.009055
MZ00005110	probable RIC1 protein {Neurospora crassa;}	0.009062
MZ00035379	NA	0.009064
MZ00024605	Mago nashi protein homolog (Mago nashi-like protein). {Oryza sativa;}	0.009074
MZ00019506	NA	0.009080
MZ00013344	Protein transport protein SEC61 gamma subunit. {Oryza sativa;}	0.009097
MZ00005312	unknown protein {Oryza sativa }	0.009111
MZ00022618	NA	0.009125
MZ00031084	OSJNBa0084K01.22 {Oryza sativa }	0.009130

MZ00034285	Ki1 protein {Beta vulgaris;}	0.009138
MZ00015537	probable cytochrome P450 monooxygenase {Zea mays}	0.009154
MZ00051002	hypothetical protein similar to Arabidopsis thaliana chromosome 5	0.009172
MZ00018942	putative cyclin-dependent kinase inhibitor {Oryza sativa }	0.009193
MZ00030324	cell division protein ftsH (ftsH)-like {Oryza sativa }	0.009197
MZ00023397	calmodulin-like {Oryza sativa }	0.009198
MZ00017649	NA	0.009219
MZ00036189	elongation factor 1 alpha {Zea mays}	0.009220
MZ00004748	GTP-binding protein - {Arabidopsis thaliana}	0.009279
MZ00044791	Similar to protein arginine N-methyl transferase 1 (Q63009) {Oryza sativa }	0.009289
MZ00036455	NA	0.009290
MZ00032566	unknown protein {Oryza sativa }	0.009291
MZ00056908	Similar to leucoanthocyanidin dioxygenase.(AI440611) {Oryza sativa }	0.009302
MZ00001737	hypothetical protein F3O9.7 - {Arabidopsis thaliana}	0.009316
MZ00031059	hypothetical protein {Oryza sativa }	0.009338
MZ00000826	hypothetical protein {Oryza sativa }	0.009342
MZ00043557	NA	0.009344
MZ00056188	NA	0.009345
MZ00044336	NA	0.009362
MZ00028881	BETL3 protein {Zea mays}	0.009369
MZ00023641	Thioredoxin H-type (TRX-H) (Phloem sap 13 kDa protein-1). {Oryza sativa;}	0.009377
MZ00048136	amino acid transporter-like {Oryza sativa }	0.009385
MZ00018201	hypothetical protein At2g01070 [imported] - {Arabidopsis thaliana}	0.009387
MZ00039969	putative target of myb1 {Oryza sativa }	0.009398
MZ00044486	gamma-tubulin 1 {Zea mays}	0.009401
MZ00005082	NA	0.009410
MZ00022793	unknown protein {Oryza sativa }	0.009424
MZ00014643	DAG protein homolog F18F4.120 - {Arabidopsis thaliana}	0.009426
MZ00043496	plastidic cysteine synthase 1 {Solanum tuberosum;}	0.009429

MZ00017757	putative ATP-dependent transporter {Oryza sativa }	0.009439
MZ00023517	NA	0.009450
MZ00036646	unknown protein {Arabidopsis thaliana}	0.009451
MZ00029058	At2g46220/T3F17.13 {Arabidopsis thaliana}	0.009452
MZ00043139	NA	0.009452
MZ00022488	unknown protein {Oryza sativa }	0.009453
MZ00048685	Unknown protein {Oryza sativa }	0.009470
MZ00043996	Bax inhibitor-1 (BI-1) (OsBI-1). {Oryza sativa;}	0.009494
MZ00022137	TGF-beta receptor-interacting protein-like protein {Oryza sativa }	0.009508
MZ00031370	NA	0.009513
MZ00005961	NA	0.009525
MZ00035841	thaumatin-like protein. {Zea mays}	0.009527
MZ00027122	unknown protein {Oryza sativa }	0.009548
MZ00028327	putative Acyl-CoA independent ceramide synthase {Oryza sativa }	0.009555
MZ00024414	putative MATE efflux family protein {Oryza sativa }	0.009558
MZ00026457	unknown protein {Oryza sativa }	0.009575
MZ00033338	AT4g38500/F20M13_60 {Arabidopsis thaliana}	0.009579
MZ00038360	putative SLT1 protein (ion homeostasis related protein) {Oryza sativa }	0.009580
MZ00026115	MADS box protein {Zea mays}	0.009594
MZ00056250	NA	0.009595
MZ00013904	ATP synthase gamma chain, mitochondrial precursor {Ipomoea batatas}	0.009615
MZ00024888	putative cellular retinaldehyde-binding/triple function {Oryza sativa }	0.009619
MZ00030258	hypothetical protein {Oryza sativa }	0.009623
MZ00031158	hypothetical protein {Oryza sativa }	0.009625
MZ00020763	NA	0.009626
MZ00018944	putative beta-glucosidase aggregating factor {Oryza sativa }	0.009641
MZ00003954	probable senescence-related protein [imported] - {Arabidopsis thaliana}	0.009650
MZ00005835	putative pectinesterase {Oryza sativa }	0.009660
MZ00019078	putative malonyl-CoA:Acyl carrier protein transacylase {Arabidopsis thaliana}	0.009666

MZ00022864	NA	0.009672
MZ00015965	TaWIN2-like protein {Oryza sativa }	0.009674
MZ00040170	NA	0.009682
MZ00056308	NA	0.009693
MZ00042346	NA	0.009699
MZ00048513	hypothetical protein At2g04740 [imported] - {Arabidopsis thaliana}	0.009700
MZ00031279	putative cullin 3B {Oryza sativa }	0.009700
MZ00004611	NA	0.009703
MZ00004760	putative HD-zip transcription factor {Oryza sativa }	0.009747
MZ00044578	vicilin-like storage protein Glb1-L, embryo - {Zea mays}	0.009747
MZ00025787	senescence-associated protein-like {Oryza sativa }	0.009748
MZ00019509	putative HEN1 {Oryza sativa }	0.009758
MZ00036881	NA	0.009759
MZ00040133	unknown protein {Oryza sativa }	0.009777
MZ00035731	putative senescence-associated protein {Pisum sativum;}	0.009782
MZ00016212	putative tyrosine-tRNA ligase {Oryza sativa }	0.009833
MZ00042295	Similar to Arabidopsis thaliana chromosome 2 BAC clone T06D20	0.009838
MZ00020737	unknown protein {Arabidopsis thaliana}	0.009840
MZ00018981	putative ZF-HD homeobox protein {Oryza sativa }	0.009863
MZ00039726	NA	0.009865
MZ00049042	serine/threonine protein kinase {Oryza sativa }	0.009875
MZ00057280	NA	0.009876
MZ00003584	OSJNBa0014K14.6 {Oryza sativa }	0.009881
MZ00051849	hypothetical protein {Oryza sativa }	0.009881
MZ00038257	NA	0.009897
MZ00005741	putative serine/threonine protein kinase {Oryza sativa }	0.009903
MZ00052435	ATPase inhibitor, mitochondrial precursor. {Saccharomyces cerevisiae}	0.009909
MZ00038302	NA	0.009912
MZ00017835	putative transposase {Oryza sativa }	0.009916

MZ00025382	NA	0.009917
MZ00042052	putative WRKY transcription factor 20 {Oryza sativa }	0.009939
MZ00005776	hypothetical protein {Oryza sativa }	0.009961
MZ00043339	Thiazole biosynthetic enzyme 1-2, chloroplast precursor.	0.009965
MZ00041700	unknown protein {Oryza sativa }	0.009975
MZ00041043	Phosphoenolpyruvate carboxykinase [ATP] (EC 4.1.1.49) {Zea mays}	0.009975

Supplementary Table 2. Differentially expressed genes in CO354 after *Fusarium verticillioides* infection.		
ID	**Putative Annotation**	**p-value**
MZ00032601	S-locus receptor-like kinase RLK11 {Oryza sativa}	4.63E-09
MZ00028434	unknown {Arabidopsis thaliana}	4.85E-09
MZ00032633	putative peroxidase {Oryza sativa}	6.09E-09
MZ00056441	putative serine/threonine kinase -related protein {Oryza sativa}	9.56E-09
MZ00035841	thaumatin-like protein. {Zea mays}	4.96E-08
MZ00012745	putative cytochrome P450 {Lolium rigidum}	7.16E-08
MZ00055869	NA	8.11E-08
MZ00028333	allele:Hd1 {Oryza sativa}	0.000158
MZ00024413	EREBP-like protein {Oryza sativa}	0.000159
MZ00032865	hypothetical protein similar to Arabidopsis thaliana chromosome 3, At3g56840 {Oryza sativa}	0.000164
MZ00038111	Zein-alpha precursor (19 kDa) (Clone 19D1). {Zea mays}	0.000166
MZ00001757	putative myosin heavy chain-like protein {Oryza sativa}	0.000168
MZ00023408	putative histone H2A {Oryza sativa}	0.000168
MZ00052294	putative RNA-binding protein {Oryza sativa}	0.000192
MZ00036152	NA	0.000204
MZ00023245	NA	0.000208
MZ00055364	At5g22340 {Arabidopsis thaliana;}	0.000223
MZ00036366	NA	0.000229
MZ00018255	calcyclin binding protein-like {Oryza sativa}	0.000279
MZ00037479	glutathione S-transferase GST 41 {Zea mays}	0.000329
MZ00020576	NA	0.000340
MZ00042818	histone H3 (clone pH3c-1) - alfalfa {Medicago sativa } histone H3 - wheat {Triticum aestivum}	0.000343
MZ00036287	Actin-depolymerizing factor 5 (ADF-5) (AtADF5). {Arabidopsis thaliana}	0.000357
MZ00014713	TGF-beta receptor-interacting protein-like protein {Oryza sativa}	0.000365
MZ00035150	NA	0.000365
MZ00004532	NA	0.000384

MZ00041327	Bowman-Birk type trypsin inhibitor (WTI). {Triticum aestivum}	0.000387
MZ00005317	NA	0.000396
MZ00017086	multi resistance protein [imported] {Arabidopsis thaliana}	0.000400
MZ00021619	unnamed protein product; gene_id:MXH1.10 unknown protein {Arabidopsis thaliana}	0.000407
MZ00027505	unknown protein {Oryza sativa}	0.000416
MZ00047119	OSJNBa0053B21.9 {Oryza sativa}	0.000438
MZ00039002	26S proteasome regulatory particle triple-A ATPase subunit2b {Oryza sativa}	0.000452
MZ00048618	hypothetical protein {Oryza sativa}	0.000532
MZ00004720	O-methyltransferase ZRP4 (EC 2.1.1.-) {Zea mays}	0.000535
MZ00027099	glutathione S-transferase GST 11 {Zea mays}	0.000546
MZ00042199	unknown protein {Oryza sativa}	0.000555
MZ00035282	roothairless 1 {Zea mays}	0.000564
MZ00017736	putative glycine rich protein {Oryza sativa}	0.000653
MZ00013414	NA	0.000668
MZ00023590	NA	0.000689
MZ00036267	putative glucosyl transferase {Sorghum bicolor}	0.000694
MZ00037579	Zein-alpha precursor (19 kDa) {Zea mays}	0.000728
MZ00027263	Similar to putative Ser/Thr protein kinase (AC003105) {Oryza sativa}	0.000733
MZ00018447	NA	0.000755
MZ00037421	OSJNBa0040D17.13 {Oryza sativa}	0.000835
MZ00042047	putative senescence-associated protein 12 {Arabidopsis thaliana}	0.000848
MZ00003886	OSJNBa0029H02.14 {Oryza sativa}	0.000852
MZ00034836	Cytochrome b6-f complex subunit 4 (17 kDa polypeptide). {Zea mays}	0.000863
MZ00027442	protein F27F5.5 [imported] {Arabidopsis thaliana}	0.000875
MZ00014653	unknown protein {Oryza sativa}	0.000897
MZ00022671	putative auxin-responsive GH3 protein {Oryza sativa}	0.000900
MZ00052022	IscA -like {Oryza sativa}	0.000909
MZ00038882	NA	0.000910
MZ00006883	unknown protein {Oryza sativa}	0.000912

MZ00039490	40S ribosomal protein S27 homolog {Zea mays}	0.000931
MZ00022365	At5g38880 {Arabidopsis thaliana}	0.000973
MZ00057416	transposase related protein {Zea mays}	0.001017
MZ00042881	putative 60S ribosomal protein L37a {Oryza sativa}	0.001021
MZ00007505	unknown protein {Oryza sativa}	0.001043
MZ00013680	hypothetical protein similar to Arabidopsis thaliana chromosome 3	0.001043
MZ00036487	Ubiquitin-like protein SMT3. {Oryza sativa}	0.001044
MZ00003440	NA	0.001059
MZ00017764	putative chloride channel protein {Oryza sativa}	0.001066
MZ00039981	putative cellular apoptosis susceptibility protein.	0.001096
MZ00003543	putative receptor-like protein kinase 4 {Oryza sativa}	0.001121
MZ00013864	acyl carrier protein. {Zea mays}	0.001139
MZ00036162	rRNA promoter binding protein {Rattus norvegicus}	0.001140
MZ00013991	hypothetical protein F21B7.14 - Arabidopsis thaliana {Arabidopsis thaliana}	0.001160
MZ00039058	type IIB calcium ATPase MCA5 {Medicago truncatula}	0.001165
MZ00036295	unnamed protein product; no similarity, hypothetical start {Kluyveromyces lactis}	0.001197
MZ00017997	putative elicitor inducible beta-1,3-glucanase {Oryza sativa}	0.001219
MZ00005581	expressed protein {Oryza sativa}	0.001220
MZ00039347	polyubiquitin-like protein [imported] - Arabidopsis thaliana {Arabidopsis thaliana}	0.001232
MZ00004700	putative metacaspase, having alternative splicing products {Oryza sativa}	0.001259
MZ00038094	zein {Zea mays}	0.001260
MZ00012632	SPX (SYG1/Pho81/XPR1) domain-containing protein-like {Oryza sativa}	0.001266
MZ00013626	Malate dehydrogenase, cytoplasmic (EC 1.1.1.37). {Zea mays}	0.001276
MZ00016972	alpha-expansin 1 {Zea mays}	0.001276
MZ00036315	Photosystem I reaction centre subunit N, chloroplast precursor (PSI-N). {Hordeum vulgare}	0.001284
MZ00021606	putative non-phototropic hypocotyl 3 (NPH3) {Oryza sativa}	0.001285
MZ00035063	NA	0.001311
MZ00005067	kinesin-like protein {Oryza sativa}	0.001354
MZ00041832	putative parathymosin {Oryza sativa}	0.001390

MZ00021334	NA	0.001397
MZ00031330	OSJNBa0064G10.19 {Oryza sativa}	0.001409
MZ00034978	unknown protein {Oryza sativa}	0.001416
MZ00035502	glyceraldehyde-3-phosphate dehydrogenase {Vaccinium myrtillus}	0.001427
MZ00036046	putative lipase {Oryza sativa}	0.001430
MZ00042689	NA	0.001461
MZ00022946	NA	0.001498
MZ00031599	importin-beta1 {Oryza sativa}	0.001519
MZ00036530	unnamed protein product {Kluyveromyces lactis}	0.001520
MZ00036307	Putative ubiquitin-conjugating enzyme {Arabidopsis thaliana}	0.001523
MZ00013430	elongation factor {Triticum aestivum}	0.001536
MZ00032337	NA	0.001537
MZ00055517	PHD finger protein-like {Oryza sativa}	0.001558
MZ00032417	NA	0.001579
MZ00028436	hypothetical protein {Arabidopsis thaliana}	0.001605
MZ00049711	putative pentatricopeptide (PPR) repeat-containing protein {Oryza sativa}	0.001623
MZ00039962	NA	0.001631
MZ00038863	unknown protein {Oryza sativa}	0.001636
MZ00021093	hypothetical protein {Oryza sativa}	0.001636
MZ00018438	NA	0.001650
MZ00057083	putative serine/threonine-protein kinase ctr1 {Oryza sativa}	0.001687
MZ00015167	putative asparaginyl-tRNA synthetase, chloroplast/mitochondrial precursor {Oryza sativa}	0.001694
MZ00028634	hypothetical protein {Oryza sativa}	0.001718
MZ00015994	expressed protein {Oryza sativa}	0.001742
MZ00030179	SET domain-containing protein SET118 {Zea mays}	0.001750
MZ00031422	unknown protein {Oryza sativa}	0.001771
MZ00028909	NA	0.001783
MZ00038610	NA	0.001786
MZ00023755	gibberellin response modulator {Zea mays}	0.001791

MZ00026687	putative UVB-resistance protein UVR8 {Oryza sativa}	0.001847
MZ00055012	putative far-red impaired response protein {Oryza sativa}	0.001860
MZ00044170	unknown protein {Oryza sativa}	0.001923
MZ00032428	NA	0.001985
MZ00027741	putative histidine-rich Ca2+-binding protein {Oryza sativa}	0.002009
MZ00043461	P0076O17.9 {Oryza sativa}	0.002075
MZ00041696	NA	0.002085
MZ00028716	unknown protein {Oryza sativa}	0.002088
MZ00019218	NA	0.002093
MZ00014693	OSJNBa0041A02.17 {Oryza sativa}	0.002109
MZ00020986	NA	0.002173
MZ00020486	unknown protein {Oryza sativa}	0.002192
MZ00029194	OSJNBa0079A21.10 {Oryza sativa}	0.002200
MZ00041964	RacB protein {Zea mays}	0.002212
MZ00024426	NA	0.002261
MZ00040154	NA	0.002262
MZ00039950	hypothetical protein At2g27230 Arabidopsis thaliana bHLH transcription factor	0.002270
MZ00016241	WD-40 repeat protein-like {Oryza sativa}	0.002286
MZ00038366	NA	0.002292
MZ00037150	NA	0.002305
MZ00044584	NA	0.002322
MZ00042988	ADP-ribosylation factor 1 {Oryza sativa}	0.002348
MZ00028014	chromosome condensation protein-like {Oryza sativa}	0.002363
MZ00024854	putative mono- or diacylglycerol acyltransferase {Oryza sativa}	0.002379
MZ00032142	NA	0.002391
MZ00007245	putative m6A methyltransferase {Oryza sativa}	0.002441
MZ00048168	hypothetical protein [imported] {Arabidopsis thaliana}	0.002461
MZ00035864	NA	0.002463
MZ00047197	putative integral membrane protein {Oryza sativa}	0.002463

MZ00048395	putative ABC transporter {Oryza sativa}	0.002465
MZ00056217	unknown protein {Arabidopsis thaliana}	0.002482
MZ00035287	putative RPT2 {Oryza sativa}	0.002487
MZ00031627	unknown protein {Oryza sativa}	0.002518
MZ00051816	putative senescence-associated protein 15 {Oryza sativa}	0.002520
MZ00048352	OSJNBa0014K14.4 {Oryza sativa}	0.002522
MZ00015765	putative translation initiation factor 5A {Oryza sativa}	0.002554
MZ00005780	OSJNBa0040D17.13 {Oryza sativa}	0.002558
MZ00052386	NA	0.002558
MZ00025306	RAB1Y {Lotus corniculatus}	0.002582
MZ00032537	cytochrome P450 {Oryza sativa}	0.002585
MZ00020150	OSJNBb0054B09.4 {Oryza sativa}	0.002587
MZ00057395	putative potasium transporter {Oryza sativa}	0.002601
MZ00025897	myosin-like protein {Arabidopsis thaliana}	0.002603
MZ00037026	putative CCR4-NOT transcription complex subunit 7 {Oryza sativa}	0.002607
MZ00038103	zein zA1 - maize {Zea mays}	0.002609
MZ00004737	ribosomal protein-like {Oryza sativa}	0.002648
MZ00020228	glutathione S-transferase GST 31 {Zea mays}	0.002654
MZ00020838	putative SET domain-containing protein SET104 {Oryza sativa}	0.002664
MZ00027190	unknown protein {Oryza sativa}	0.002667
MZ00016188	putative 2-oxoacid-dependent oxidase {Oryza sativa}	0.002668
MZ00036698	putative senescence-associated protein {Pisum sativum}	0.002782
MZ00021512	putative NADPH-cytochrome P450 oxydoreductase isoform 3 {Oryza sativa}	0.002782
MZ00014802	unknown protein, {Arabidopsis thaliana}	0.002795
MZ00005090	Protein transport protein SEC61 beta subunit. {Yarrowia lipolytica}	0.002822
MZ00041513	wound-induced protease inhibitor {Zea mays}	0.002829
MZ00036544	probable cytochrome P450 monooxygenase {Zea mays}	0.002864
MZ00023556	NA	0.002891
MZ00001422	NA	0.002895

MZ00013437	60S ribosomal protein L17. {Zea mays}	0.002897
MZ00005951	contains EST D23238(C2469) kinase-like protein {Oryza sativa}	0.002923
MZ00019427	nitrate reductase {Zea mays}	0.002930
MZ00056774	putative pumilio-family RNA-binding domain-containing protein(PPD1) {Oryza sativa}	0.002949
MZ00029063	NA	0.002960
MZ00023730	histone H4 {Arabidopsis thaliana}	0.002975
MZ00034916	Phosphinothricin N-acetyltransferase (EC 2.3.1.-) {Streptomyces hygroscopicus}	0.003001
MZ00021181	hypothetical protein {Arabidopsis thaliana}	0.003046
MZ00048481	rice EST AU030811, similar to rice Ca+2-ATPase (U82966) {Oryza sativa}	0.003047
MZ00046836	unknown protein {Oryza sativa}	0.003055
MZ00017719	putative fatty acyl coA reductase {Oryza sativa}	0.003077
MZ00006993	hypothetical protein {Oryza sativa}	0.003083
MZ00029828	NA	0.003111
MZ00005928	NA	0.003123
MZ00055581	unknown protein {Oryza sativa}	0.003149
MZ00013226	putative lipase {Oryza sativa}	0.003174
MZ00025937	putative 2-hydroxyacid dehydrogenases family protein {Oryza sativa}	0.003177
MZ00019245	CDK5RAP3-like protein. {Arabidopsis thaliana}	0.003188
MZ00028425	putative tetrafunctional protein of glyoxysomal fatty acid beta-oxidation {Oryza sativa}	0.003198
MZ00038967	BRI1-KD interacting protein 116 {Oryza sativa}	0.003209
MZ00024202	RNA-binding protein-like {Oryza sativa}	0.003220
MZ00029191	Similar to Lupinus luteus glutaminyl -tRNA synthetase {Oryza sativa}	0.003221
MZ00044501	DNAJ homologue {Oryza sativa}	0.003241
MZ00036129	NA	0.003253
MZ00044395	Bronze-2 protein - maize {Zea mays}	0.003268
MZ00027809	growth-regulating factor 1 {Oryza sativa}	0.003291
MZ00024484	17.8 kDa class II heat shock protein. {Zea mays}	0.003294
MZ00035965	hypothetical protein {Oryza sativa}	0.003321
MZ00022040	NA	0.003327

MZ00052169	putative kinase interacting protein {Oryza sativa}	0.003332
MZ00018209	shrunken seed protein (SSE1) {Arabidopsis thaliana}	0.003340
MZ00055826	NA	0.003342
MZ00014560	probable germin protein 4 - rice {Oryza sativa}	0.003358
MZ00022409	hypothetical protein {Oryza sativa}	0.003367
MZ00022517	NA	0.003398
MZ00038772	putative copper-exporting ATPase {Oryza sativa}	0.003410
MZ00005766	NA	0.003439
MZ00014105	Similar to PNIL34. (U37437) {Oryza sativa}	0.003467
MZ00039273	NA	0.003477
MZ00040744	unknown protein {Oryza sativa}	0.003490
MZ00017082	DNA topoisomerase homolog F8F16.30 {Arabidopsis thaliana}	0.003505
MZ00031259	TAF11 {Arabidopsis thaliana}	0.003514
MZ00017599	putative protein kinase. {Oryza sativa}	0.003523
MZ00038505	unknown protein {Oryza sativa}	0.003542
MZ00051949	NA	0.003564
MZ00055877	hypothetical protein {Oryza sativa}	0.003565
MZ00022432	putative proton-dependent oligopeptide transport {Oryza sativa}	0.003567
MZ00024029	OSJNBb0051N19.2 {Oryza sativa}	0.003577
MZ00013112	Phospholipid-transporting ATPase 1 (EC 3.6.3.1) {Arabidopsis thaliana}	0.003618
MZ00026774	putative acetoacetyl-coenzyme A thiolase {Oryza sativa}	0.003631
MZ00015077	hypothetical protein {Oryza sativa}	0.003638
MZ00016573	hypothetical protein {Oryza sativa}	0.003638
MZ00042213	NA	0.003645
MZ00014573	putative mitochondrial processing peptidase alpha subuunit {Oryza sativa}	0.003646
MZ00013952	zinc finger and C2 domain protein-like {Oryza sativa}	0.003658
MZ00014805	chlorophyll a/b-binding protein CP26 precursor {Zea mays}	0.003668
MZ00036603	seven transmembrane protein Mlo8 {Zea mays}	0.003694
MZ00016604	U2 snRNP auxiliary factor, small subunit {Oryza sativa}	0.003716

MZ00020159	NA	0.003747
MZ00022238	NA	0.003758
MZ00054995	NA	0.003763
MZ00037230	unknown protein {Oryza sativa}	0.003796
MZ00034372	OSJNBb0103I08.18 {Oryza sativa}	0.003800
MZ00031794	NA	0.003813
MZ00048280	putative WD40 protein Ciao1 {Oryza sativa}	0.003826
MZ00004386	putative ZmEBE-1 protein {Oryza sativa}	0.003870
MZ00016937	putative protein {Arabidopsis thaliana}	0.003874
MZ00040526	DNA-directed RNA polymerase beta subunit {Cuscuta reflexa}	0.003879
MZ00038211	Glyceraldehyde 3-phosphate dehydrogenase, cytosolic 3 (EC 1.2.1.12). {Zea mays}	0.003883
MZ00024574	NADH dependent Glutamate Synthase {Oryza sativa}	0.003885
MZ00003581	pentatricopeptide (PPR) repeat-containing protein-like protein {Oryza sativa}	0.003893
MZ00031520	unknown protein {Oryza sativa}	0.003921
MZ00029808	potential U2 snRNA pseudouridine synthase-like {Oryza sativa}	0.003926
MZ00043537	Similar to Spinacia oleracea protein kinase {Oryza sativa}	0.003946
MZ00021172	hydroxyproline-rich glycoprotein DZ-HRGP {Volvox carteri}	0.003949
MZ00000389	NA	0.003961
MZ00034276	NA	0.003973
MZ00055433	unknown {Arabidopsis thaliana}	0.003992
MZ00022567	putative hydrolase {Oryza sativa}	0.004020
MZ00021681	NA	0.004030
MZ00023269	NA	0.004048
MZ00035440	unknown protein {Oryza sativa}	0.004051
MZ00040893	putative hUPF2 {Oryza sativa}	0.004063
MZ00004553	putative DNA-binding protein {Oryza sativa}	0.004071
MZ00035844	unnamed protein product	0.004095
MZ00028652	unknown protein {Oryza sativa}	0.004124
MZ00037194	NA	0.004134

MZ00039865	hypothetical protein {Arabidopsis thaliana}	0.004188
MZ00035489	NA	0.004213
MZ00042752	unknown protein {Oryza sativa}	0.004218
MZ00056341	NA	0.004224
MZ00026511	bHLH transcription factor-like protein {Oryza sativa}	0.004247
MZ00043459	P0076O17.9 {Oryza sativa}	0.004271
MZ00006911	unknown protein {Oryza sativa}	0.004282
MZ00004215	putative NAC domain protein NAC1 {Oryza sativa}	0.004321
MZ00005352	NA	0.004324
MZ00032893	OSJNBa0086O06.20 {Oryza sativa}	0.004340
MZ00043267	succinate dehydrogenase subunit 3 {Oryza sativa}	0.004347
MZ00015935	putative embryogenic callus protein 98b {Oryza sativa}	0.004350
MZ00033336	putative zinc finger protein {Oryza sativa}	0.004424
MZ00037745	bundle sheath cell specific protein 1 {Zea mays}	0.004425
MZ00017988	hypothetical protein similar to Arabidopsis thaliana chromosome 3, At3g17930 {Oryza sativa}	0.004426
MZ00005759	hypothetical protein F14I3.4 [imported] {Arabidopsis thaliana}	0.004485
MZ00052061	auxin-induced protein-like {Oryza sativa}	0.004485
MZ00001544	molybdenum cofactor sulfurase protein -like {Oryza sativa}	0.004490
MZ00031884	pentatricopeptide (PPR) repeat-containing protein-like {Oryza sativa}	0.004493
MZ00042517	aldehyde oxidase (EC 1.2.3.1) 2 - maize {Zea mays}	0.004498
MZ00017317	unknown {Triticum monococcum;}	0.004499
MZ00001750	Phospholipase D alpha 2 {Oryza sativa}	0.004521
MZ00038659	hypothetical protein F2K15.100 {Arabidopsis thaliana}	0.004551
MZ00027569	unknown protein {Arabidopsis thaliana}	0.004571
MZ00020520	putative 28 kDa Golgi SNARE protein {Oryza sativa}	0.004574
MZ00048688	putative placental protein 6 {Oryza sativa}	0.004579
MZ00035263	glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) {Zea mays}	0.004589
MZ00020259	beta3-glucuronyltransferase {Zea mays}	0.004595
MZ00041899	zein {Zea mays}	0.004597

MZ00003713	respiratory burst oxidase protein B {Oryza sativa}	0.004606
MZ00038119	Aspartate aminotransferase, chloroplast precursor (EC 2.6.1.1) {Arabidopsis thaliana}	0.004635
MZ00027108	putative DD1A protein {Oryza sativa}	0.004636
MZ00027471	NA	0.004667
MZ00004862	Fruiting body protein SC3 precursor {Schizophyllum commune}	0.004677
MZ00018043	putative plastidic ATP/ADP-transporter {Oryza sativa}	0.004679
MZ00041980	probable beta-keto acyl reductase - maize {Zea mays}	0.004680
MZ00037011	NA	0.004683
MZ00030552	unknown protein {Oryza sativa}	0.004699
MZ00042179	putative sugar transporter protein {Zea mays}	0.004711
MZ00055111	OJ991113_30.6 {Oryza sativa}	0.004714
MZ00029535	picA protein. {Oryza sativa}	0.004722
MZ00019613	NA	0.004740
MZ00001488	NA	0.004763
MZ00031444	translocon Tic40 {Pisum sativum}	0.004788
MZ00040182	NA	0.004816
MZ00038067	putative cytochrome P450 {Oryza sativa}	0.004827
MZ00043527	tonoplast membrane integral protein ZmTIP3-1 {Zea mays}	0.004827
MZ00022687	T6D22.15 {Arabidopsis thaliana}	0.004831
MZ00036211	Hypothetical UPF0195 protein At1g68310. {Arabidopsis thaliana}	0.004860
MZ00048241	putative PWWP domain protein {Oryza sativa}	0.004865
MZ00024230	putative protein disulfide isomerase {Oryza sativa}	0.004905
MZ00037574	NA	0.004910
MZ00005205	NA	0.004917
MZ00030883	ribosomal protein L2 {Zea mays}	0.004928
MZ00032152	unknown protein {Oryza sativa}	0.004935
MZ00001014	chlorophyll a/b binding protein {Oryza sativa}	0.004965
MZ00024617	putative chorismate mutase precursor {Oryza sativa}	0.004967
MZ00003766	O-methyltransferase ZRP4 (EC 2.1.1.-) {Zea mays}	0.004981

MZ00027606	unknown protein {Oryza sativa}	0.004983
MZ00018555	OSJNBb0061C13.16 {Oryza sativa}	0.004991
MZ00051207	putative oxygenase {Oryza sativa}	0.005017
MZ00014271	circadian oscillator component {Oryza sativa}	0.005023
MZ00029310	hypothetical protein T8M16_160 {Arabidopsis thaliana}	0.005030
MZ00049496	hypothetical protein {Oryza sativa}	0.005034
MZ00029033	NA	0.005081
MZ00029932	At3g26990 {Arabidopsis thaliana}	0.005099
MZ00055496	OSJNBa0070C17.22 {Oryza sativa}	0.005110
MZ00035310	isocitrate dehydrogenase (NADP+) {Eucalyptus globulus}	0.005142
MZ00018370	putative AP endonuclease {Sorghum bicolor}	0.005165
MZ00055146	contains EST C26242(C11933) unknown protein {Oryza sativa}	0.005207
MZ00031181	fatty acid alpha-oxidase {Oryza sativa}	0.005226
MZ00035073	NA	0.005250
MZ00039481	cytochrome reductase {Triticum aestivum}	0.005259
MZ00055132	unknown protein {Arabidopsis thaliana}	0.005268
MZ00050476	putative nodulin 3 {Oryza sativa}	0.005277
MZ00013616	Alpha-1,4-glucan-protein synthase (EC 2.4.1.112) {Zea mays}	0.005281
MZ00014888	putative z-protein {Oryza sativa}	0.005283
MZ00041188	NA	0.005291
MZ00000793	NA	0.005294
MZ00033393	OSJNBa0067K08.2 {Oryza sativa}	0.005298
MZ00022693	hypothetical protein {Oryza sativa}	0.005317
MZ00048443	putative fatty acid elongase {Oryza sativa}	0.005355
MZ00039660	contains EST AU096093(S12136) unknown protein {Oryza sativa}	0.005356
MZ00015332	sucrase-like protein {Oryza sativa}	0.005370
MZ00018040	NA	0.005392
MZ00013203	OSJNBa0058K23.4 {Oryza sativa}	0.005395
MZ00032481	OSJNBa0088A01.18 {Oryza sativa}	0.005420

MZ00043913	putative DNA primase large subunit {Oryza sativa}	0.005439
MZ00013541	putative 60S ribosomal protein L28 {Oryza sativa}	0.005456
MZ00012622	putative carboxylate oxidase {Oryza sativa}	0.005481
MZ00033772	NA	0.005491
MZ00036482	putative photosystem II subunit (22KDa) precursor {Oryza sativa}	0.005501
MZ00021294	putative sugar transporter protein {Zea mays}	0.005533
MZ00020455	OSJNBa0065B15.1 {Oryza sativa}	0.005561
MZ00031937	mitochondrial uncoupling protein 1 {Saccharum officinarum}	0.005567
MZ00020796	putative chaperonin CPN60-2, mitochondrial precursor {Oryza sativa}	0.005594
MZ00036700	expressed protein, having alternative splicing products {Oryza sativa}	0.005611
MZ00026133	putative cleavage and polyadenylation specifity factor {Oryza sativa}	0.005635
MZ00019598	hypothetical protein F24I3.170 - Arabidopsis thaliana {Arabidopsis thaliana}	0.005645
MZ00020296	unknown protein {Oryza sativa}	0.005652
MZ00039476	At3g08610 {Arabidopsis thaliana}	0.005663
MZ00001876	hypothetical protein similar to Arabidopsis thaliana chromosome 2, At2g19870	0.005668
MZ00036860	NA	0.005702
MZ00023373	histone H3. {Helicoverpa zea}	0.005717
MZ00031078	putative PPR-repeat containing protein {Oryza sativa}	0.005729
MZ00014581	putative cytochrome P450 {Oryza sativa}	0.005753
MZ00016975	putative transmembrane protein(TPA regulated locus protein) {Oryza sativa}	0.005769
MZ00033577	NA	0.005786
MZ00023086	OSJNBa0059D20.8 {Oryza sativa}	0.005789
MZ00030960	NA	0.005810
MZ00057024	putative oxidoreductase, FAD-binding {Oryza sativa}	0.005823
MZ00018513	OSJNBa0040D17.15 {Oryza sativa}	0.005825
MZ00023901	calcineurin B-like {Oryza sativa}	0.005825
MZ00027871	Unknown protein {Oryza sativa}	0.005840
MZ00035672	OSJNBb0034I13.15 {Oryza sativa}	0.005853
MZ00015118	unknown protein {Oryza sativa}	0.005857

MZ00027971	NA	0.005867
MZ00022045	NA	0.005875
MZ00056476	putative HGA1 {Oryza sativa}	0.005884
MZ00012874	unknown protein {Oryza sativa}	0.005894
MZ00023425	Eukaryotic translation initiation factor 5A {Zea mays}	0.005942
MZ00042969	NA	0.005977
MZ00041326	putative Bowman-Birk serine protease inhibitor {Oryza sativa}	0.005995
MZ00004466	NA	0.006000
MZ00046727	expressed protein {Oryza sativa}	0.006006
MZ00030403	putative Mannosyl-oligosaccharide 1,2-alpha-mannosidase IB {Oryza sativa}	0.006010
MZ00001314	putative auxin response factor {Oryza sativa}	0.006018
MZ00038914	NA	0.006022
MZ00032363	unknow protein {Oryza sativa}	0.006046
MZ00039890	putative cinnamoyl CoA reductase {Oryza sativa}	0.006093
MZ00045593	putative pol protein {Zea mays}	0.006094
MZ00048746	putative embryogenesis-associated protein {Oryza sativa}	0.006095
MZ00003820	putative lateral organ boundaries (LOB) domain protein 37 {Oryza sativa}	0.006107
MZ00016415	putative dermal glycoprotein precursor {Oryza sativa}	0.006121
MZ00001090	NA	0.006129
MZ00027161	putative nuclear protein p30 {Oryza sativa}	0.006166
MZ00052434	MADS box interactor-like {Oryza sativa}	0.006216
MZ00001033	VHS1 protein {Oryza sativa}	0.006235
MZ00035598	hypothetical protein At2g19370 {Arabidopsis thaliana}	0.006256
MZ00025712	hypothetical protein {Oryza sativa}	0.006284
MZ00055664	NA	0.006297
MZ00025733	putative zinc finger protein {Oryza sativa}	0.006353
MZ00037746	putative senescence-associated protein {Pisum sativum}	0.006354
MZ00055081	putative phenylalanine ammonia-lyase {Oryza sativa}	0.006368
MZ00021899	NA	0.006370

MZ00032868	OSJNBa0018M05.8 {Oryza sativa}	0.006372
MZ00015090	putative MATE family protein {Oryza sativa}	0.006391
MZ00041386	putative TPR repeat containing protein {Oryza sativa}	0.006403
MZ00055373	NA	0.006404
MZ00003603	NA	0.006410
MZ00004150	proline-rich protein M14 precursor {Mus musculus}	0.006416
H300015051	NA	0.006430
MZ00004728	NA	0.006431
MZ00040190	NA	0.006438
MZ00023482	putative ribosomal protein S29 {Oryza sativa}	0.006454
MZ00028399	putative peroxidase {Oryza sativa}	0.006476
MZ00057406	Gag and Pol {Zea mays}	0.006497
MZ00050557	unknown protein {Oryza sativa}	0.006541
MZ00041938	putative 15.9 kDa subunit of RNA polymerase II {Oryza sativa}	0.006546
MZ00043875	hypothetical protein {Oryza sativa}	0.006551
MZ00030866	NA	0.006556
MZ00056079	NA	0.006571
MZ00056418	OSJNBa0004N05.3 {Oryza sativa}	0.006593
MZ00037237	voltage-dependent anion channel protein 2 {Zea mays}	0.006597
MZ00031430	hypothetical protein {Oryza sativa}	0.006597
MZ00037650	22K zein precursor (clone ZSF4C1) {Zea mays}	0.006604
MZ00015523	KNOX family class 2 homeodomain protein {Oryza sativa}	0.006610
MZ00038699	putative Aconitate hydratase {Oryza sativa}	0.006616
MZ00028857	NA	0.006626
MZ00035288	putative splicing factor-like protein {Oryza sativa}	0.006633
MZ00039964	OSJNBb0034I13.8 {Oryza sativa}	0.006648
MZ00028298	putative mitochondrial carrier protein {Sorghum bicolor}	0.006664
MZ00007440	putative Splicing factor, arginine/serine-rich 7 {Oryza sativa}	0.006673
MZ00020350	oxidation resistance 1-like protein {Oryza sativa}	0.006695

MZ00027985	Ras-related protein Rab11C. {Arabidopsis thaliana}	0.006701
MZ00018658	OSJNBb0004A17.4 {Oryza sativa}	0.006702
MZ00032503	expressed protein {Oryza sativa}	0.006703
MZ00023520	NA	0.006704
MZ00028705	hypothetical protein {Oryza sativa}	0.006732
MZ00021367	putative serine carboxypeptidase {Oryza sativa}	0.006735
MZ00003777	putative anthocyanin 5-O-glucosyltransferase {Oryza sativa}	0.006775
MZ00040173	NA	0.006803
MZ00025432	NA	0.006820
MZ00019128	hypothetical protein {Oryza sativa}	0.006834
MZ00052087	putative beta-D-galactosidase {Oryza sativa}	0.006836
MZ00013005	putative nucleic acid-binding protein {Oryza sativa}	0.006845
MZ00030398	NA	0.006860
MZ00045680	NA	0.006868
MZ00042180	putative tocopherol polyprenyltransferase {Oryza sativa}	0.006872
MZ00029291	hypothetical protein similar to Arabidopsis thaliana chromosome 4, At4g04870	0.006879
MZ00035730	Profilin A. {Oryza sativa}	0.006913
MZ00016134	putative NF-E2 inducible protein {Oryza sativa}	0.006915
MZ00023260	putative transcription factor X1 {Oryza sativa}	0.006917
MZ00001706	putative chloroplast nucleoid DNA-binding protein cnd41 {Oryza sativa}	0.006922
MZ00036186	unnamed protein product; no similarity, hypothetical start {Kluyveromyces lactis}	0.006940
MZ00039287	Zein-alpha precursor (19 kDa) {Zea mays}	0.006946
MZ00026353	OSJNBa0086O06.9 {Oryza sativa}	0.006958
MZ00024711	Probable xyloglucan endotransglucosylase/hydrolase precursor {Triticum aestivum}	0.006976
MZ00022017	Similar to Arabidopsis thaliana DNA chromosome 4, ESSA I AP2 contig;	0.006976
MZ00042688	NA	0.006989
MZ00038818	NA	0.007000
MZ00028354	ribosomal protein S10 {Zea mays}	0.007007
MZ00043007	19kD alpha zein D1 {Zea mays}	0.007018

MZ00007227	Probable polyadenylate-binding protein At2g36660 {Arabidopsis thaliana}	0.007023
MZ00017133	putative auxin-regulated protein {Oryza sativa}	0.007024
MZ00048832	RNA binding protein {Homo sapiens}	0.007039
MZ00014363	Bowman-Birk type wound induced proteinase inhibitor WIP1 precursor. {Zea mays}	0.007043
MZ00027551	hypothetical protein F25L23.250 {Arabidopsis thaliana}	0.007089
MZ00040938	NA	0.007113
MZ00036203	NA	0.007118
MZ00028088	unknown protein {Oryza sativa}	0.007122
MZ00046815	Dehydration responsive element binding protein 2F {Arabidopsis thaliana}	0.007130
MZ00017642	DNA binding protein-like {Oryza sativa}	0.007131
MZ00018736	Putative bHLH transcription protein {Oryza sativa}	0.007180
MZ00029852	putative thiamin pyrophosphokinase {Oryza sativa}	0.007195
MZ00026457	unknown protein {Oryza sativa}	0.007208
MZ00022224	bHLH transcription factor-like protein {Oryza sativa}	0.007216
MZ00021145	phytochromobilin synthase {Zea mays}	0.007247
MZ00035787	unnamed protein product {Kluyveromyces lactis}	0.007256
MZ00005751	NA	0.007271
MZ00039442	Putative transporter {Oryza sativa}	0.007278
MZ00034977	putative nin one binding protein {Oryza sativa}	0.007279
MZ00021167	putative HAK2 (K+ transporter) {Oryza sativa}	0.007314
MZ00015814	unknown protein {Oryza sativa}	0.007315
MZ00030215	kinesin heavy chain-like protein {Oryza sativa}	0.007316
MZ00035291	putative GDP dissociation inhibitor {Oryza sativa}	0.007322
MZ00015027	terpene synthase 5 {Zea mays}	0.007327
MZ00031179	hydroxyproline-rich glycoprotein DZ-HRGP {Volvox carteri}	0.007436
MZ00025308	OSJNBa0055C08.14 {Oryza sativa}	0.007447
MZ00048780	unknown protein {Oryza sativa}	0.007458
MZ00029568	Unknown protein {Oryza sativa}	0.007463
MZ00039909	putative DNA-binding protein, 5'-partial {Oryza sativa}	0.007469

MZ00039798	OSJNBa0083N12.1 {Oryza sativa}	0.007474
MZ00031061	NA	0.007478
MZ00020081	OSJNBa0014K14.7 {Oryza sativa}	0.007478
MZ00007546	CSLC9 {Oryza sativa}	0.007493
MZ00020894	putative metallophosphatase {Lupinus luteus}	0.007498
MZ00029387	NA	0.007498
MZ00056628	copper chaperone {Populus alba x Populus tremula}	0.007505
MZ00032926	putative polyprotein {Oryza sativa}	0.007509
MZ00038988	NA	0.007511
MZ00039701	thioredoxin family-like protein {Oryza sativa}	0.007520
MZ00030032	OSJNBa0019K04.8 {Oryza sativa}	0.007537
MZ00048883	B1340F09.23 {Oryza sativa}	0.007565
MZ00043034	NA	0.007571
MZ00041308	F28N24.7 unknown protein {Oryza sativa}	0.007573
MZ00031707	NA	0.007591
MZ00040923	putative 60S ribosomal protein L44 {Oryza sativa}	0.007599
MZ00036223	putative senescence-associated protein {Pisum sativum}	0.007613
MZ00003643	unknown protein {Oryza sativa}	0.007621
MZ00020131	spore coat protein -like {Oryza sativa}	0.007654
MZ00019763	NA	0.007660
MZ00039286	zein {Zea mays}	0.007668
MZ00023527	NA	0.007673
MZ00035506	NA	0.007704
MZ00004556	hypothetical protein At2g31610 {Arabidopsis thaliana}	0.007707
MZ00046947	putative CCR4-associated factor 1 {Oryza sativa}	0.007720
MZ00001607	centromere protein-like {Oryza sativa}	0.007746
MZ00019127	hypothetical protein {Oryza sativa}	0.007752
MZ00038551	putative RNA helicase {Arabidopsis thaliana}	0.007753
MZ00025888	unknown {Arabidopsis thaliana}	0.007774

MZ00044727	NA	0.007809
MZ00021452	NA	0.007851
MZ00015707	translation initiation factor IF-3-like {Oryza sativa}	0.007856
MZ00032649	NA	0.007860
MZ00016830	putative biotin synthase {Oryza sativa}	0.007890
MZ00052272	NA	0.007891
MZ00016206	mRNA capping enzyme - like protein {Arabidopsis thaliana}	0.007894
MZ00016046	putative 40S ribosomal protein S15A {Oryza sativa}	0.007907
MZ00021327	OSJNBa0086O06.15 {Oryza sativa}	0.007908
MZ00055191	NA	0.007944
MZ00005204	NA	0.007955
MZ00036048	putative respiratory burst oxidase protein {Oryza sativa}	0.007956
MZ00039069	putative diphosphate-fructose-6-phosphate 1-phosphotransferase alpha chain {Oryza sativa}	0.007964
MZ00035067	NA	0.007977
MZ00028924	putative permease {Oryza sativa}	0.007980
MZ00057204	putative glucose-6-phosphate/phosphate- translocator precursor {Oryza sativa}	0.007980
MZ00015473	putative hexose transporter {Oryza sativa}	0.007984
MZ00018504	OSJNBb0065L13.3 {Oryza sativa}	0.007989
MZ00023892	chlorophyll a/b-binding protein (cab-m7) precursor {Zea mays}	0.007989
MZ00014093	putative steroid membrane binding protein {Oryza sativa}	0.008011
MZ00039187	transcriptional factor B3-like {Oryza sativa}	0.008019
MZ00003575	NA	0.008044
MZ00035731	putative senescence-associated protein {Pisum sativum}	0.008066
MZ00016340	cytochrome P450 monooxygenase CYP72A16 {Zea mays}	0.008089
MZ00001613	proton-exporting ATPase {Cucumis sativus}	0.008104
MZ00057295	TRAB1 {Oryza sativa}	0.008111
MZ00026671	NA	0.008114
MZ00026177	Hypothetical protein {Oryza sativa}	0.008153
MZ00024724	putative nuclease I {Oryza sativa}	0.008157

MZ00031126	unknown protein {Oryza sativa}	0.008161
MZ00025274	calcineurin B-like protein 9 {Arabidopsis thaliana}	0.008186
MZ00041828	NADH-dependent glyceraldehyde-3-phosphate dehydrogenase {Oryza sativa}	0.008191
MZ00048651	chloride channel {Oryza sativa}	0.008199
MZ00022348	NA	0.008222
MZ00020391	contains EST AU032660(S13119) unknown protein {Oryza sativa}	0.008245
MZ00048631	Putative indole-3-acetate beta-glucosyltransferase {Oryza sativa}	0.008250
MZ00023783	OSJNBb0116K07.11 {Oryza sativa}	0.008311
MZ00040302	NA	0.008312
MZ00041330	putative translation initiation factor eIF-2 beta chain {Oryza sativa}	0.008317
MZ00021757	NA	0.008321
MZ00037974	zp22/D87 {Zea mays}	0.008321
MZ00020243	AT3g52870/F8J2_40 {Arabidopsis thaliana}	0.008331
MZ00030787	putative nucleotide excision repair protein XP-D {Oryza sativa}	0.008351
MZ00029357	putative MtN3 {Oryza sativa}	0.008354
MZ00030902	NA	0.008365
MZ00018981	putative ZF-HD homeobox protein {Oryza sativa}	0.008375
MZ00018622	MLM24.13 unknown protein {Arabidopsis thaliana}	0.008376
MZ00035663	NA	0.008391
MZ00036918	CDH1-D {Gallus gallus;}	0.008396
MZ00018670	F15D2.24 unknown protein {Oryza sativa}	0.008413
MZ00044177	unknown protein {Oryza sativa}	0.008420
MZ00013400	putative HGA1 {Oryza sativa}	0.008428
MZ00022253	expressed protein {Oryza sativa}	0.008441
MZ00055056	MGC83213 protein {Xenopus laevis;}	0.008466
MZ00003675	hypothetical protein similar to Arabidopsis thaliana chromosome 5, At5g18750	0.008504
MZ00004252	putative UDP-glucosyltransferase {Oryza sativa}	0.008507
MZ00026670	NA	0.008525
MZ00033472	OSJNBb0085C12.17 {Oryza sativa}	0.008535

MZ00028026	unknown protein {Arabidopsis thaliana}	0.008549
MZ00004440	NA	0.008550
MZ00041274	putative thioredoxin peroxidase {Oryza sativa}	0.008553
MZ00021208	NA	0.008560
MZ00022514	putative outward rectifying potassium channel {Oryza sativa}	0.008561
MZ00040817	putative WD repeat protein {Oryza sativa}	0.008564
MZ00005776	hypothetical protein {Oryza sativa}	0.008600
MZ00004797	NA	0.008606
MZ00013575	unknown protein {Oryza sativa}	0.008615
MZ00025325	putative signal recognition particle 68K protein {Oryza sativa}	0.008618
MZ00031189	putative genetic modifier {Oryza sativa}	0.008619
MZ00016444	putative cystathionine beta-lyase {Oryza sativa}	0.008622
MZ00031593	putative inner membrane protein {Oryza sativa}	0.008625
MZ00029863	NA	0.008635
MZ00014895	signal peptidase protein-like protein {Cucumis melo}	0.008698
MZ00050480	putative ring finger protein {Oryza sativa}	0.008704
MZ00050523	putative calcium-dependent protein kinase {Oryza sativa}	0.008718
MZ00038092	19kD alpha zein B1 {Zea mays}	0.008746
MZ00005359	NA	0.008771
MZ00019160	putative transposase {Oryza sativa}	0.008772
MZ00020498	NA	0.008774
MZ00023866	inorganic diphosphatase (EC 3.6.1.1), H+-translocating, vacuolar membrane {Oryza sativa}	0.008778
MZ00020423	OSJNBb0042I07.2 {Oryza sativa}	0.008785
MZ00055779	NA	0.008790
MZ00048452	Similar to hexose carrier protein HEX6 &RCCHCP_1 {Oryza sativa}	0.008805
MZ00004869	NA	0.008825
MZ00025911	hypothetical protein {Oryza sativa}	0.008839
MZ00056071	NA	0.008865
MZ00048478	putative wall-associated serine/threonine kinase {Oryza sativa}	0.008871

MZ00014618	OSJNBa0067K08.8 {Oryza sativa}	0.008878
MZ00019387	unknown protein {Oryza sativa}	0.008896
MZ00043111	putative small nuclear ribonucleoprotein polypeptide G {Oryza sativa}	0.008964
MZ00029097	putative Splicing factor 3B subunit 3 {Oryza sativa}	0.008966
MZ00006990	putative ribosomal protein {Oryza sativa}	0.009001
MZ00044692	F10B6.3 {Arabidopsis thaliana}	0.009031
MZ00034441	NA	0.009062
MZ00024756	GTP cyclohydrolase I {Lycopersicon esculentum}	0.009072
MZ00042854	putative ribosomal protein L19 {Oryza sativa}	0.009088
MZ00048269	hypothetical protein {Oryza sativa}	0.009099
MZ00029178	NA	0.009100
MZ00028281	tonoplast membrane integral protein ZmTIP1-2 {Zea mays}	0.009118
MZ00040531	hypothetical protein 25 - maize mitochondrion (type N) {Zea mays}	0.009119
MZ00038819	NA	0.009128
MZ00040336	probable amino acid permease {Arabidopsis thaliana}	0.009133
MZ00019633	putative sentrin-specific protease {Oryza sativa}	0.009159
MZ00017750	NA	0.009182
MZ00001446	peroxisomal membrane protein OsPex14p {Oryza sativa}	0.009240
MZ00017253	unknown protein {Arabidopsis thaliana}	0.009264
MZ00013228	NA	0.009265
MZ00043264	putative RNA helicase {Oryza sativa}	0.009268
MZ00022199	probable peroxidase {Oryza sativa }	0.009284
MZ00056561	putative cytochrome P450 {Oryza sativa}	0.009309
MZ00021565	hypothetical protein {Oryza sativa}	0.009326
MZ00014055	putative late embryogenesis abundant protein {Oryza sativa}	0.009334
MZ00024536	protein kinase CK2 regulatory subunit CK2B3 {Zea mays}	0.009348
MZ00022315	probable zinc-finger protein {Arabidopsis thaliana}	0.009377
MZ00015865	putative protein kinase AFC3 {Oryza sativa}	0.009377
MZ00000472	NA	0.009383

MZ00024051	putative hydroxyproline-rich glycoprotein {Oryza sativa}	0.009402
MZ00029292	OSJNBa0058K23.18 {Oryza sativa}	0.009406
MZ00027727	NA	0.009420
MZ00017657	putative MADS-domain transcription factor {Zea mays}	0.009427
MZ00003585	unknown protein {Oryza sativa}	0.009455
MZ00012562	putative protein {Arabidopsis thaliana}	0.009475
MZ00029390	NA	0.009486
MZ00046774	alpha-expansin OsEXPA23 {Oryza sativa}	0.009501
MZ00037631	putative prolyl-tRNA synthetase {Oryza sativa}	0.009520
MZ00043160	NA	0.009524
MZ00021752	putative potyviral helper component protease-interacting protein {Oryza sativa}	0.009530
MZ00042691	putative MAP kinase kinase {Oryza sativa}	0.009542
MZ00027560	unnamed protein product; hypothetical protein {Oryza sativa}	0.009556
MZ00055379	NA	0.009626
MZ00043792	putative LRR receptor-like protein kinase {Oryza sativa}	0.009638
MZ00016446	NA	0.009643
MZ00039488	NA	0.009671
MZ00024135	dTDP-glucose 4,6-dehydratase {Zea mays}	0.009686
MZ00017526	magnesium transporter CorA-like {Oryza sativa}	0.009695
MZ00001128	NA	0.009718
MZ00023898	probable DNA-binding protein GBP16 - rice {Oryza sativa}	0.009739
MZ00035157	MADS box protein 1 {Zea mays}	0.009766
MZ00027938	putative adenyl cyclase {Oryza sativa}	0.009780
MZ00038252	ubiquinol-cytochrome-c reductase-like protein {Arabidopsis thaliana}	0.009786
MZ00030103	putative arm repeat containing protein {Oryza sativa}	0.009793
MZ00043888	putative stearoyl-acyl carrier protein desaturase {Oryza sativa}	0.009800
MZ00038030	19K zein precursor (clone ZG31A) {Zea mays}	0.009801
MZ00029468	NA	0.009807
MZ00029776	OSJNBa0053K19.20 {Oryza sativa}	0.009818

MZ00015210	putative UDP-glucose:glycoprotein glucosyltransferase {Oryza sativa}	0.009822
MZ00047855	isoamylase N-terminal domain containing protein {Oryza sativa}	0.009846
MZ00000425	putative nck-associated protein 1 (NAP 1)	0.009847
MZ00021709	NA	0.009857
MZ00056925	OJ000114_01.12 {Oryza sativa}	0.009879
MZ00016286	putative sugar transporter {Oryza sativa}	0.009895
MZ00013604	hypothetical protein {Oryza sativa }	0.009938

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