

**BIOCHEMICAL & MOLECULAR DEFENSE
RESPONSES TRIGGERED BY ACIBENZOLAR
S-METHYL AND A MULTIPLE-ELICITOR
PLANT FORMULATION IN TOMATO**

FERNANDA CARVALHO LOPES DE MEDEIROS

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como parte das exigências do Programa de Pós-
graduação em Agronomia área de concentração em
Fitopatologia, para a obtenção do título de
“Doutora”.

Orientador
Mário Lúcio Vilela de Resende, PhD

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APROVADA em 08 de junho de 2009.

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LAVRAS
MINAS GERAIS - BRASIL

A minha querida mãe

e aos meus irmãos Fábio e Mara,

que incondicionalmente, me apoiaram sempre.

OFEREÇO

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GENERAL ABSTRACT

MEDEIROS, Fernanda Carvalho Lopes de. **Biochemical & molecular defense responses triggered by acibenzolar S-methyl and a multiple-elicitor plant formulation in tomato.** 2009. 135p. Thesis (Doctor in Phytopathology) – Federal University of Lavras, Lavras, MG.*

Undertaken major effort in plant biochemical research is to elucidate the defense signalling pathways in plants leading to resistance to plant pathogens and potentially triggered by elicitors. Hence, the analysis of gene expression profiles in response to an elicitor molecule treatment provided the basis to identify common and/or antagonistic features among defense pathways. To date, no data was published on the effect of natural formulations on genes expression profiles but earlier studies showed activation of PR-proteins and cell wall reinforcement as effective, suggesting that plant changes due to a natural formulation elicitor, might be involved in the induced defense. However, most previous work suggesting a defensive role for natural formulations provided data about downstream events (e.g., PR protein activity), without utilizing molecular techniques to reveal changes in gene expression levels. Our goal was to assess the role of a multiple-elicitor plant formulation based on coffee leaf (NEFID) in the induction of plant defense responses by probing defensive pathways at genomic and metabolic levels and comparing the responses to acibenzolar-S-methyl, a commercial inducer. In addition, we aimed to identify a set of candidate genes that would be regulated by the natural formulation and use these obtained results to better understand the molecular mechanisms underlying tomato induced resistance. For this purpose, tomato plants were treated with the elicitors, challenged or not with *Xanthomonas vesicatoria*, the disease severity, growth, leaf area were evaluated and the mechanisms activated were assessed, such as PR-1 and β -1,3- glucanase gene expression pattern, levels of PR proteins (chitinase and β -1,3-glucanase), lignin deposition, polyphenol-oxidase and peroxidase activities as well as the overall tomato gene expression using microarray approaches. Plantlets treated with NEFID demonstrated 35% disease severity reduction, a significantly reduction (61%) was obtained by ASM-treated plants. This severity reduction appears to be based on a rapid β -1,3-glucanase and PR-1 gene expression increase (12 hours after spraying) followed by an early response in a chitinase, glucanase and polyphenol oxidase activities (24 hours after spraying) and a high lignin deposition on leaf tissue. A total

*Guidance Committee: Mário Lúcio V. de Resende – UFLA,
Paul W. Paré – Texas Tech University

of 268 genes had changed regulation due to NEFID spraying, compared with water-treated control, with a majority of up-regulated transcripts which encoded mainly signal transduction, defense-related, oxidative burst and transcription factor genes. Since no evidence for salicylic acid or jasmonic acid buildup was found but mitogen activated proteins (MAP3K and MAPKK) as well as calcium dependent (calmodulin and phosphatidylinositol) signaling molecules were found, a SA-independent PR accumulation is likely to occur. The PRs chitinase, glucanase and peroxidase, with direct reported activity on pathogens, were up-regulated and they were not likely to suffer post-translational regulation since the corresponding enzyme activities were over-expressed as early as 24h after treatment and eventually remained as such for up to five days onward (glucanase and peroxidase). Therefore, the studied plant formulation represent a potential broad spectrum disease control.

Key-words: microarray, SAR, PR-1, chitinase, β -1,3-glucanase, peroxidase, RT-PCR, natural formulation, ASM

RESUMO GERAL

MEDEIROS, Fernanda Carvalho Lopes de. **Respostas de defesa bioquímicas e moleculares ativadas por Acibenzolar S-metil e por uma formulação natural a base de múltiplos eliciadores em tomateiro.** 2009. 135p. Tese (Doutorado em Fitopatologia) – Universidade Federal de Lavras, Lavras, MG.*

Esforços têm sido mobilizados para elucidar as rotas de defesa em plantas que levam à resistência a fitopatógenos, potencialmente ativadas pelo tratamento com eliciadores. Neste sentido, a análise da expressão de genes em resposta ao tratamento com moléculas eliciadoras representa uma ferramenta para identificar características comuns e/ou das rotas de defesa. Até o presente, nenhum dado foi publicado sobre o efeito de formulações naturais no perfil da expressão gênica, mas estudos anteriores mostraram a ativação de proteínas relacionadas à patogênese (PRs) e um potencial reforço da parede celular, sugerindo que as mudanças em plantas devidas a um eliciador, baseado em formulação natural, pode estar envolvido na defesa induzida. Contudo, as respostas raramente são correlacionadas a parâmetros de crescimento ou, feita uma abordagem multiplex para estudo não apenas de produtos de início de rota como aqueles de final. O objetivo deste trabalho foi avaliar o papel de uma formulação baseada em extrato de folha de café (NEFID) na indução de respostas de defesa de plantas, pelo monitoramento de rotas de defesa nos níveis genômicos e metabólicos e, compará-lo ao indutor comercial acibenzolar-S-metil. Objetivou-se também o estudo de genes candidatos regulados pela formulação natural (NEFID) para melhor entender os mecanismos moleculares envolvidos na resistência induzida de tomate. Para este fim, plantas tratadas com os eliciadores foram desafiadas ou não com *Xanthomonas vesicatoria* e avaliados a severidade da doença, o crescimento e área foliar bem como os mecanismos de ativação da resposta de defesa, avaliando-se o padrão de expressão de PR-1 e glucanase, os níveis de proteínas PR (quitinase e β -1,3-glucanase), deposição de lignina, atividades de oxidase de polifenol e peroxidase assim como o perfil geral da expressão de genes de tomate usando a técnica do microarranjo. As plantas tratadas com NEFID demonstraram 35% de redução na severidade da doença e redução de 61% foi encontrada em plantas tratadas com ASM. Esta redução na severidade foi relacionada ao rápido aumento da ativação de β -1,3-glucanase e PR-1 (12 horas após tratamento) seguida por uma resposta rápida da atividade de quitinase, glucanase, polifenoloxidase (24 horas após tratamento) e deposição de lignina. Um total de 268 genes tiveram regulação

*Comitê Orientador: Mário Lúcio V. de Resende – UFLA,
Paul W. Paré – Texas Tech University.

mudada devido ao tratamento com NEFID, comparado com a testemunha tratada com água, com a maioria dos genes transcritos super-expressos, os quais codificaram principalmente para transdução de sinal, defesa, estresse oxidativo e fatores de transcrição. Uma vez que nenhuma evidência para o acúmulo de ácido salicílico ou jasmônico foi encontrada, mas proteínas quinases ativadas por mitogênicos (MAP3K e MAPKK) assim como proteínas sinalizadoras dependentes de cálcio (calmodulina e fosfatidilinositol) foram encontradas, é provável que esteja ocorrendo um acúmulo de PR independente de ácido salicílico. As PRs, quitinase, glucanase e peroxidase, com atividade direta relatadas sobre patógenos, foram super-expressas e elas provavelmente não sofreram regulação pós-transcricional, uma vez que a atividade destas enzimas foi super-expressa logo às 24h após o tratamento e eventualmente permaneceram assim por pelo menos mais cinco dias (glucanase e peroxidase). Portanto, a formulação a base do extrato de planta estudado representa um potencial para o controle de um amplo espectro de doenças.

Palavras-chave: microarranjo, SAR, PR-1, quitinase, β -1,3-glucanase, peroxidase, RT-PCR, formulação natural, ASM

CHAPTER 1

1 GENERAL INTRODUCTION

Tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* Miller) is an economically important crop worldwide, and a preeminent model system for genetic studies in plants (Barone et al., 2007). Bacterial leaf spot is caused by the bacterium *Xanthomonas vesicatoria* in tomato (*Lycopersicon esculentum*) and infection results in a decreased yield at harvest. Control of the disease is difficult, often requiring expensive and complex integrated pest management (IPM), including the use of contamination-free seeds, sanitization practices, and the use of chemicals (Araújo et al., 2003).

Plants are able to survive an attack by a potential pathogen when prior treated with elicitors of defense responses, reacting with a local and systemic induction of a succession of defenses that prevent or contain the infection and provide enhanced resistance to subsequent infections by the same or even unrelated pathogens (Montesano et al., 2003). Plants can also induce defense reactions to a broad range of pathogens as a result of prior exposure to pathogens or physical stress (Metraux, 2001). The ability of plants to react to an invader by triggering local and systemic responses was explained by the production of a signal released from the infected leaf and translocated to other parts of the plant where it induces defense reactions, known as systemic acquired resistance (SAR). SAR underlines the ability of plants to acquire a state of general resistance after an initial infection (Metraux, 2001).

However some plant extracts can be made up of multiple elicitors. An natural formulation from coffee leaves (NEFID) is likely to have multiple plant and microbe-derived elicitors and has proven to be effective against multiple pathogens. The natural formulation protected tomato plants against bacterial

pathogen. Cotton plants sprayed with NEFID had a reduction in the severity of bacterial blight and this result was similar to ASM (Ishida et al., 2007)The product has also been effective for the control of coffee rust and phoma spot (Barguil et al., 2005; Santos et al., 2007).

To evaluate the role of a natural formulation based on *Coffea arabica* leaves or acibenzolar-S-methyl in triggering induced resistance, tomato plants were treated with the elicitors, challenged or not with *Xanthomonas vesicatoria* and the mechanisms activated were evaluated, such as levels of PR proteins (chitinase and β -1,3-glucanase), lignin deposition, polyphenol-oxidase and peroxidase activities as well as the overall tomato gene expression using microarray approaches.

2 LITERATURE REVIEW

This chapter presents an overview of defense responses and the microarray approach to identify a set of candidate genes to better understand the molecular and biochemical mechanisms underlying induced resistance.

2.1 Aspects of induced resistance to pathogens

In nature, plants are continuously exposed to pathogens and the preventive elicitation for resistance helps those facing further attacks. Plants respond to pathogens by activating a variety of defense mechanisms. These defense responses include hypersensitive programmed cell death (Dangl et al., 1996; Greenberg, 1997), induction of defense or defense-related genes (Dixon & Harrison, 1990), cross-linking and reinforcement of cell walls (Brisson et al., 1994), biosynthesis of phytoalexins and metabolism of phenolic compounds (Nicholson & Hammerschmidt, 1992), and production of active oxygen species, such as singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radical (Lamb & Dixon, 1997; Bolwell, 1999). A hypersensitive response at the site of infection is often manifested as necrotic lesions resulting from host cell death (Staskawicz et al., 1995; Dempsey et al., 1999). In the distal uninfected parts of the plant, systemic acquired resistance develops to provide long-lasting broad spectrum resistance against pathogens.

2.2 The mechanisms involved in systemic acquired resistance

2.2.1 Enzymes involved in the induction of resistance:

a. Peroxidases

A class III plant peroxidase (POX, EC 1.11.1.7) is a glycoprotein that catalyzes oxidoreduction between H₂O₂ and various reductants (Hiraga et al., 2000).

Many reports have suggested that POXs play roles in resistance to pathogens such as lignification and suberization (Dean & Kolattukudy, 1976, Quiroga et al., 2000), cross-linking of cell wall proteins (Showalter, 1993), xylem wall thickening (Hilaire et al., 2001), generation of reactive oxygen species (Bestwick et al., 1998), hydrogen peroxide scavenging (Kawaoka et al., 2003), phytoalexin synthesis (Kristensen et al., 1999), antifungal activity of POX itself (Caruso et al., 2001) and auxin metabolism (Lagrimini et al., 1997). However, it is difficult to define the specific functions of individual POXs because of their low substrate specificity in vitro and the presence of many isoenzymes (Hiraga et al., 2000).

Some *pox* genes are activated by infection with pathogens such as fungi (Thordal-Christensen et al., 1992, Harrison et al., 1995, Curtis et al., 1997), bacteria (Young et al., 1995, Bestwick et al., 1998), viruses (Lagrimini and Rothstein 1987, Hiraga et al., 2000b) and viroids (Vera et al., 1993). Therefore, it is classified to the pathogenesis-related (PR) protein-9 family (van Loon et al., 1994).

Many studies have indicated the importance of POXs in defense against pathogen infection. On tomato plants infected with *Xanthomonas vesicatoria*, POX activity accumulated when plants were elicited with a plant formulation or the commercial product acibenzolar-S-methyl as early as 4h after treatment and its expression remained steady until 72h after treatment (AT), the last sampled time point (Cavalcanti et al., 2006). The enzyme was also reported as part of the resistance against fungal infection. Plants treated with 2,6-dichloroisonicotinic acid (INA) and challenged with powdery mildew accumulated peroxidase in a fast manner until 3 days AT and kept it steadily up

until 15 days AT. The induction of the scavenger enzyme was accompanied by an increase in β -1,3-glucanase and, plants treated with the elicitor and subsequently treated with diphenylene iodonium, an inhibitor of the oxidative burst, had a reduced level of not only POX but also β -1,3-glucanase activities. The same authors observed that plants treated with H₂O₂ expressed high levels of the scavenger but no expression of the PR, suggesting that the peroxidase induced upon elicitation is part of a defense network to hinder the pathogen development (Kang, 2009).

b. Polyphenol oxidases

Polyphenol oxidases (PPOs; EC 1.14.18.1 or EC 1.10.3.2) are nuclear-encoded enzymes of almost ubiquitous distribution in plants (Mayer & Harel, 1979; Mayer, 1987). PPOs catalyze the oxygen-dependent oxidation of phenols to quinones.

Because of their conspicuous reaction products and their wound and pathogen inducibilities, PPOs have been suggested to participate in plant defense against pests and pathogens (Mayer & Harel, 1979; Mayer, 1987; Steffens et al., 1994; Constabel et al., 1995; Thipyapong et al., 1995; Thipyapong & Steffens, 1997). Systemic induction of PPO expression in response to wounding and pathogens might provide an additional line of defense to protect plants against further attack by pathogen and insects (Bashan et al., 1987; Constabel et al., 1995; Thipyapong et al., 1995; Stout et al., 1999).

The activity of PPO is also of broad spectrum, acting in the control of fungal and bacterial pathogens (Cavalcanti et al., 2007; Daw et al., 2008). The accumulation of polyphenol oxidase was associated with peach resistance to decay when treated with methyl jasmonate, a well-known plant defense elicitor (Jin et al., 2009). The PPO activity has also been reported in plants treated with

natural formulations also showing disease resistance (Cavalcanti et al., 2007; Daw et al., 2008).

Down regulation of all the members of the PPO gene family was observed when antisense PPO cDNA was introduced in tomato plants and examined the resistance of the plants to the pathogen *Pseudomonas syringae*. PPO activity was reduced by a factor of about 40. Examination of the sensitivity of the plants to the pathogen revealed a dramatic increase in their susceptibility, although the overall growth and development of the tomato plants was not affected by the down regulation of PPO (Thipyapong et al., 2004).

In other experiment in which PPO was over-expressed in tomato plants (Li & Steffens, 2002), the over-expression was accompanied by enhanced resistance to the same pathogen (*P. syringae*). The levels of mRNA rose to a much greater extent than the levels of PPO protein. These findings clearly implicate PPO in the defense of plants against pathogens but do not as yet provide an explanation of the underlying mechanism.

c. Glucanases

The glucanases (β -1,3-endoglucanases; EC 3.2.1.6) are present in a wide variety of plants, animals and microorganisms (Jwanny et al., 2001).

Plant β -1,3-glucanases are referred as PR-2 proteins and are subdivided into three classes. Class I glucanases are basic proteins of about 33 kDa and are localized in the plant vacuole (Bulcke et al., 1989). Classes II and III include acidic, extracellular proteins of about 36 kDa (Theis and Stahl, 2004). They participate in several physiological and developmental plant processes. In addition, class I β -1,3-glucanases exhibit antifungal activity both *in vitro* and *in planta* (Joshi et al., 1998; Mauch et al., 1988). Class II β -1,3-

glucanases exhibit *in vitro* antifungal activity only if applied in combination with chitinases or class I β -1,3-glucanases (Theis and Stahl, 2004).

d. Chitinases

Chitinases (EC 3.2.1.14) constitute the second largest group of antifungal proteins. They catalyse the hydrolytic cleavage of the β -1,4-glycoside bond present in biopolymers of N-acetyl-D-glucosamine producing chito-oligosaccharides of 2–6 N-acetyl-D-glucosamine residues in length (Stintzi et al., 1993).

Chitinases are classified in families 18 and 19 of the 57 families in which O-glycoside hydrolases are presently subdivided (Henrissat and Bairoch, 1996). Higher plants synthesize seven different classes of chitinases which differ in protein structure, substrate specificity, mechanism of catalysis and sensitivity to inhibitors (Brunner et al., 1998). These classes are grouped into three families of PR proteins (Neuhaus et al., 1996): chitinases of classes Ia, Ib, II, IV, VI and VII belong to the PR-3 family, whereas those of classes III and V are included in the PR-8 and PR-11 families, respectively. Additionally, some proteins with low endochitinase activity occur in the PR-4 family (chitin-binding proteins) (Melchers et al., 1994). Acidic chitinases belonging to classes Ib, II, III, IV and VI are secreted to the apoplast, whereas basic chitinases included in classes Ia, III and VI are located in vacuoles (Arie et al., 2000).

Chitinases have been reported to play a role in growth and development such as vegetative storage protein (Peumans et al., 2002), antifreeze activity (Yeh et al., 2000), aspartic protease and α -amylase inhibitor activity (Ary et al., 1989; Guevara et al., 1999) but its more widely reported function is on disease resistance.

Plant chitinases that hydrolyze chitin, inhibit the growth of fungi and generate chitin oligosaccharides that act as new elicitors for long-term

resistance induction (Sharp et al., 1984). In addition, many chitinases are induced by pathogen attack and some isoforms exhibit *in vitro* antifungal properties. For these reasons, chitinases are believed to play a major role in plant host defense against pathogens.

The antifungal activity displayed by many chitinases was initially assumed to derive from their ability to digest chitin, leading to a weakened fungal cell wall and subsequent cell lysis. However, recent evidence indicates that the mechanisms by which chitinases inhibit fungal growth seem to be more dependent on the presence of a chitin-binding domain than on chitinolytic activity. Thus, the antifungal activity of a tobacco class I chitinase is three times higher when a chitin-binding domain is present (Iseli et al., 1993).

Not rarely the activities of glucanases and chitinases have been reported as being operative in induced plants against pathogens (Campos et al., 2009; Cavalcanti et al., 2006) and the elicitation may be accomplished by a broad range of products, from commercial resistance inducers to plant formulations and avirulent pathogens (Campos et al., 2009; Cavalcanti et al., 2006).

An increase of up to eight fold in levels of both glucanase and chitinase were observed when non-pathogenic *Colletotrichum lindemuthianum* race Delta strains were used to treat common bean plants and the increase in the detected enzyme highly correlated with the control of anthracnose (Campos et al., 2009). The enzymes were also reported to be over-expressed in tomato plants when treated with plant resistance activators and later challenged with a bacterial pathogen (Cavalcanti et al., 2006). Both glucanases and chitinases have lysozyme activity in the hydrolysis of the $\beta(1-4)$ bonds between N-acetyl glucosaminic and muramic, which is components of the bacterial cell wall (Majeau et al., 1990).

Since the majority of pathogens are susceptible to glucanases and chitinases, plants over-expressing these pathogenesis-related proteins represent a plausible strategy to assure disease control (Honée, 1999). Furthermore, the degradation of fungal cell wall releases oligomers that are recognized by the plant and triggers broad spectrum disease resistance genes (Buchanan, 2000) effective not only against fungal pathogens but also against virus (Guo et al., 2002).

Some plants are particularly susceptible to a wide range of pathogens and its production is highly dependent on fungicide spray-based disease control. One of such is tomato, the plant has been bred for some diseases but the constitutive broad spectrum disease control has yet to be achieved. Therefore, Tabaeizadeh et al., (1999) constructed transgenic tomato lines over-expressing chitinase and glucanase from its wild relative *Lycopersicon chilense*. As a result of the engineering, plants became more resistant to vascular wilts.

However, the broad spectrum disease control by the over-expression of PR-proteins may not be an ecological acceptable technology, since transgenic plants may harm non-pathogenic fungi, some of which have a direct beneficial effect on plant growth such as mycorrhizae in increase the phosphorus up-take (Girlanda et al., 2008). Furthermore, both chitinase (Lutz et al., 2003) and glucanase (Damasceno et al., 2008) have already been reported as present among plant pathogenic fungi and by exerting a selection pressure by the only use of transgenic plants expressing those genes may lead to failure of the disease control strategy.

On the other side, glucanase and chitinase expression after exogenous elicitor treatment is generally accompanied by the induction of a series of other disease control responses such as cell wall cross-linking, lignifications, production of phenolics as mentioned in the other sections.

2.2.2 Lignification and Other Structural Barriers

Lignin is one of the most structurally complex of the biopolymers. The chemical construction of lignin a three-dimensional, branched polymer formed by the oxidative polymerization of three substituted cinnamyl alcohols: p-coumaryl alcohol (I), coniferyl alcohol (II), and sinapyl alcohol (III).

Regardless of the nature of the formed lignin in plants, evidence suggests that the esterification of polymerized phenols to cell-wall materials is strongly related to the expression of resistance (Fry, 1986; Fry, 1987). Since it accumulates rapidly following infection (Farmer, 1985, Grand et al., 1987) and has been implicated in the chemical modification of cell walls to be more resistant to its degrading enzymes, increase in the resistance of walls to the diffusion of toxins from the pathogen to the host and of nutrients from the host to the pathogen, produce toxic precursors and free radicals, and the actual lignification and entrapment of the pathogen (Ride, 1978). In fungal penetration sites, lignin is deposited to hinder the pathogen development in structural barriers such as papillae (Cadena-Gomez & Nicholson, 1987; Ride & Pearce, 1979; Vance et al., 1980).

Hence, the role of pathogen-induced lignin and related polymers has been closely correlated with the defense responses in several plants to multiple pathogens (Vance et al., 1980).

The accumulation of lignin was related to the control of soybean to *Sclerotinia sclerotiorum* in resistant cultivars (Peltier et al., 2009). This accumulation was also observed in the resistance of cotton plants to root rot pathogens.

For foliar pathogens the lignin content as well as total phenols have also been correlated with disease resistance. Reimers & Leach (1991) have

shown that the race-specific resistance of rice carrying the *Xa-10* gene to *X. oryzae* is correlated with the deposition of lignin at the site of infection. A similar finding was observed for tomato cultivars resistant to *Xanthomonas vesicatoria* (Kavitha & Umesha, 2008).

2.3 Elicitors for systemic acquired resistance

Several elicitors have been studied as triggers of systemic acquired resistance (SAR). Vallad & Goodman (2004) reviewed the commercially available ones and the obtained field results. For most of the results where it was compared to a standard control there was no observed statistical difference.

In their review the more commonly cited product was acibenzolar-S-methyl (Bion or Actiguard as trade names) a commercial product derived from benzothiadiazole (BTH), which in turn is a functional analog of salicylic acid, known to stimulate the production of plant defense-related compounds and induce systemic acquired resistance (SAR), distinguished from other plant defense responses by local and systemic activation of specific pathogenesis-related genes (PR), mentioned in detail in the previous sessions (Thaler et al., 1999; Kuc, 2001; Durrant & Dong, 2004).

Although ASM has proven to be effective against a wide range of pathogens and pests, it is particularly an attractive tool for the control of bacterial and virus disease, where effective control is hardly achieved.

Herman & Smart (2007) studied the expression level of the SAR marker gene in tomato following ASM sprays in three cultivars. While early expression of PR-1 (a marker gene of SAR) increased by up to 5 fold until 3 days after spraying (DAS), this increase was dramatically different among cultivars and peaked at different time-courses. The cultivar Rio Grande peaked 22 fold increase early in the time-course (7-8 DAS) but dropped sharply afterwards, Rutgers cultivar peaked later 9-10 DAS and dropped more

smoothly and Supersonic cultivar had a more flat bell-shaped curve similar to the one obtained in the early studied time-course.

The plant disease resistance activator does not assure 100% protection against bacterial spot of tomato in the field but is an important player in the integrated pest management. Roberts et al., (2008) obtained 12% disease control when using ASM alone but achieved up to 67% when alternating ASM sprays with copper hydroxide and mancozeb.

Many other commercial disease resistance inducers have been studied with variable efficacy (reviewed by Resende et al., 2006). A promising field of study is the prospection of leaf formulations with the potential for SAR triggering.

A suspension of *Crinipellis pernicioso* mycelium protected tomato plants against *Xanthomonas vesicatoria* by up to 87% and the main elicitor part of the preparation was mainly made up of heterologous chitosan (Cavalcanti et al., 2007).

However some plant extracts can be made up of multiple elicitors. A seaweed based extract has laminarin and carrageenans, two polysaccharides which are recognized by inespecific receptors on the plant and triggers defense related genes in tobacco (Mercier et al., 2001). Another formulation from coffee leaves (EFID) has recently been patented (I.N.P.I., Protocol number 0000220604167501, “Formulação para indução de resistência”... Aug/2006) has not been fractionated but is likely to have multiple plant and microbe-derived elicitors and has proven to be effective against multiple pathogens. The product has been effective for the control of coffee rust and phoma spot and one possible mechanisms of resistance is the buildup of lignin content in the leaves following plant sprays (Barguil et al., 2005; Santos et al., 2007).

The plant formulation also protected tomato plants against bacterial pathogen. Cotton plants sprayed with EFID had a reduction in the severity of bacterial blight and this result was similar to ASM (Ishida et al., 2007).

2.4 Functional genomics in the study of plant defense against pathogens

When a plant is treated with a plant defense activator, several responses have been elucidated but the overall changes in the plant are starting to be investigated and one important tool being used is the microarray technique.

In order to better understand the operation of the SAR pathway, using *Arabidopsis thaliana* for which a vast literature has already been generated, the genome has been fully sequenced and most genes are known, Blanco et al. (2009) identified two pathways for the activation of SAR genes could be distinguished, the NPR1 independent represent only 14% of them. These two groups of genes, not only differ in their main functional categories, but also in their timing and mechanism of activation by SA. Not only have been found defense related responses but SAR elicited plants also displayed genes involved acclimatory responses to stress, such as recovery of the cell redox balance (glutathione transferase, UDP glycosyl transferase and glutaredoxins), intracellular stress signaling, improvement of pathogen recognition, and promotion of metabolic changes. Glutaredoxins along with thioredoxins catalyze reversible thiol-based reduction of target proteins, implicated in the SA-dependent reduction of NPR1 and TGA factors required for the transcription of defense genes and ecotopic expression of a glutaredoxin gene silenced the expression of PDF1.2 suggesting that this gene may be one of the switches in the induced systemic resistance pathway (SAR/ISR).

Since early studies on SAR-associated events, Maleck et al. (2000) identified not only transcripts peculiar to this pathway but also co-regulated traits involved in redox homeostasis.

Even if the whole genome has not yet been sequenced for all plant species, many genes have known functions and several attempts are helping to better understand how commercial plants defend themselves against pathogens.

An over 33,000 citrus gene chip is available and helped Kim et al. (2009) explain what changes *Candidatus liberibacter* has on the plant in an attempt to explain the plant pathogen interactions. They found that the clogged sieved elements are made up mainly of carbohydrates and this corroborates with the leaf up-regulation of three key starch biosynthetic genes including ADP-glucose pyrophosphorylase, starch synthase, granule-bound starch synthase and starch debranching enzyme.

In a 7,883 microarray chip, customized for ESTs of rust infected soybean at different time points and deposited sequences, cultivars carrying the genes Rpp1-Rpp4 conferring resistance to *Phakopsora pachyrhizi*, 558 were found to have changed regulation among which the defense related ones represented to highest group and they mainly belonged either to peroxidases or lipoxygenases. Conversely, cell-wall-associated protein such as extensins, proline-rich proteins, and xyloglucan endotransglycosylases were found to be down-regulated (Choi et al., 2008).

Similar studies on potato gene response from cultivars resistant to late blight (*Phytophthora infestans*) using a customized cDNA chip carrying 1,009 spots, defense-related metabolic pathways such as the biosynthesis of aromatic amino acids, phenylpropanoids, oxylipin, and ethylene, were activated, which resulted in the *de novo* synthesis of salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), suggesting that a combination of both reported signaling

pathways (SAR and JA) may be involved in the defense of potato cultivars to late blight.

Another Solanaceous species, with a simpler genome organization is tomato (*Solanum lycopersicon* L.). It is diploid, with a short generation time, routine transformation technology, and availability of rich genetic and genomic resources. It has a diploid genome with 12 chromosome pairs and an estimated genome size of 950Mb encoding approximately 35,000 genes that are largely sequestered in contiguous euchromatic regions (reviewed by Barone et al., 2008). Several microarray gene chips are marketed and presently cover up to 1/3rd of the estimated size of genome (Van der Hoeven et al., 2002). The great advantage of tomato as a model system to study metabolic changes is the vast number of genus relatives it covers (potato, eggplant, tobacco and pepper) (Moore et al., 2005).

Tomato plants were challenged with fusicoccin (FC), a diterpene glucoside toxin produced by the fungus *Fusicoccum amygdali* Del. to observe defense-related genes. Pronounced changes in transcript abundance of pathogenesis-related and a conversely down-regulation of salicylic acid synthesis suggest for an induction of PRs SA-independent. The defense responses are not JA-dependent either since a down-regulation of wound responsive genes was observed. Conversely, the jasmonic acid synthesis was found to be up-regulated and this has been linked to the JA ability inhibit the biosynthesis of photosynthetic pigments and photosynthetic activity which corroborates with the predominant down-regulation of photosynthesis related genes. The SA-independent induction of PRs was assumed to occur via mitogen activated kinase and through Ca²⁺-dependent calmodulin (Frick & Schaller, 2002).

In order to control post-harvest diseases, *Cryptococcus laurentii* a biocontrol yeast was used to spray cherry tomato fruits and using microarray

technology, a changed regulation of 531 genes was observed. Defense-related genes related to the SAR-dependent PR activation (chitinase and glucanase) were observed and an already shown interplay of salicylic acid and jasmonic acid/ethylene defense was present. Interestingly, a down-regulation of ethylene synthesis has direct implications on the fast ripening and susceptibility to disease. Other defense related responses involved the up-regulation of cytochrome P450 and a down-regulation of cell wall loosening and expansion (expansin, xyloglucan endoglycosyl transferase, polygalacturonase), enzymes directly related to fungal development (Jiang et al., 2009).

To date, no data was found on the effect of plant formulations on plant global changes but earlier work (Medeiros, unpublished) showed activation of PRs and cell wall reinforcement is operative suggesting plant changes due to a plant formulation elicitor might be involved in the broad spectrum defense. Hence, the present work will probe the responses to a multiple-elicitor plant formulation based on coffee leaf on tomato.

3 SPECIFIC OBJECTIVES

Our goal was to assess the role of Acibenzolar S-Methyl and a multiple-elicitor plant formulation based on coffee leaf (NEFID) in the induction of plant defense responses by probing defensive pathways at genomic and metabolic levels. Most previous work suggesting a defensive role for natural formulations such as NEFID provided data about downstream events (e.g., PR protein activity), without utilizing molecular techniques to reveal changes in gene expression levels, no data was found on the effect of natural formulation on plant global changes. In addition, we aimed to identify a set of candidate genes that are regulated by the natural formulation and use these results obtained to better understand the molecular mechanisms underlying tomato induced resistance. Owing to the possession of knowledge of plant-bacterial interactions in tomato, this plant system are likely to provide good models for assessing the role of these products in triggering defense responses. Monitored defense responses include: severity of black spot disease after treatments with the tested formulations, levels of PR proteins (chitinase and β -1,3-glucanase), lignin deposition, polyphenol-oxidase and peroxidase activities as well as the overall tomato gene expression using microarray approaches.

4 REFERENCES

ARAÚJO, J. S. P.; ROBBS, C. F.; RIBEIRO, R. L. D. Manejo integrado de fitobacterioses de importancia economica no Brasil: parte I. **Revisão Anual de Patologia de Plantas**, Passo Fundo, v. 11, n. 1, p. 107-131, jan. 2003.

ARIE, M.; HIKICHI, K.; TAKAHASHI, K.; ESAKA, M. Characterization of a basic chitinase which is secreted by cultured pumpkin cells. **Physiologia Plantarum**, Copenhagen, v. 110, n. 2, p. 232-239, Oct. 2000.

ARY, M. B.; RICHARDSON, M.; SHEWRY, P. R. Purification and characterization of an insect alpha-amylase inhibitor/endochitinase from seeds of Job's Tears (*Coix lachryma-jobi*). **Biochimica et Biophysica Acta**, New York, v. 999, n. 3, p. 260-266, Dec. 1989.

BARGUIL, B. M.; RESENDE, M. L. V.; RESENDE, R. S.; BEZERRA JUNIOR, E. A.; SALGADO, S. L. Effect of extracts from citric biomass, rusted coffee leaves and coffee berry husks on *Phoma costarricensis* of coffee plants. **Fitopatologia Brasileira**, Fortaleza, v. 30, n. 5, p. 535-537, set./out. 2005.

BARONE, A.; CHIUSANO, M. L.; ERCOLANO, M. R.; GIULIANO, G.; GRANDILLO, S.; FRUSCIANTE, L. Structural and functional genomics of tomato. **International Journal of Plant Genomics**, Chicago, v. 2008, p. 1-12, Jan. 2008.

BASHAN, Y.; OKON, Y.; HENIS, Y. Peroxidase, polyphenoloxidase, and phenols in relation to resistance against *Pseudomonas syringae* pv. tomato in tomato plants. **Canadian Journal of Botany**, Ottawa, v. 65, n. 2, p. 366-372, Dec. 1987.

BESTWICK, C. S.; BROWN, I. R.; MANSFIELD, J. W. Localized changes in peroxidase activity accompany hydrogen peroxide generation during the development of a nonhost hypersensitive reaction in lettuce. **Plant Physiology**, Rockville, v. 118, n. 3, p. 1067-1078, Nov. 1998.

BLANCO, F.; SALINAS, P.; CECCHINI, N. M.; JORDANA, X.; HUMMELEN, P.; ALVAREZ, M. E.; HOLUIQUE, L. Early genomic responses to salicylic acid in Arabidopsis. **Plant Molecular Biology**, Dordrecht, v. 70, n. 1/2, p. 79-102, May 2009.

BOLWELL, G. P. Role of active oxygen species and NO in plant defense responses. **Current Opinion on Plant Biology**, Amsterdam, v. 2, n. 4, p. 287-294, Aug. 1999.

BOLWELL, G. P.; ROBBINS, M. P.; DIXON, R. A. Metabolic changes in elicitor-treated bean cells: enzymic responses associated with rapid changes in cell wall components. **European Journal of Biochemistry**, New York, v. 148, n. 3, p. 571-78, Jan. 1985.

BRISSON, L. F.; TENHAKEN, R.; LAMB, C. Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. **Plant Cell**, Rockville, v. 6, n. 12, p. 1703-1712, Dec. 1994.

BRUNNER, F.; STINTZI, A.; FRITIG, B.; LEGRAND, M. Substrate specificities of tobacco chitinases. **Plant Journal**, Oxford, v. 14, n. 2, p. 225-234, Apr. 1998.

BUCHANAN, B. B.; GRUISSEM, W.; JONES, R. L. **Biochemistry and molecular biology of plants**. Rockville: American Society of Plant Physiologists, 2000. 1367 p.

BULCKE, M. V.; BAUW, G.; CASTRESANA, C.; MONTAGU, M. van; VANDEKERCKHOVE, J. Characterization of vacuolar and extracellular beta (1,3)-glucanases of tobacco: evidence for a strictly compartmentalized plant defense system. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, DC, v. 86, n. 8, p. 2673-2677, Apr. 1989.

CADENA-GOMEZ, G.; NICHOLSON, R. L. Papilla formation and associated peroxidase activity: a non-specific response to attempted fungal penetration of maize. **Physiological and Molecular Plant Pathology**, London, v. 31, n. 1, p. 51-67, July 1987.

CAMPOS, A. D.; HAMPE, M. M. V.; FERREIRA, A. G.; ANTUNES, I. F.; CASTRO, L. A. S. de. Induction of systemic resistance to anthracnose in common bean by the avirulent delta race of *Colletotrichum lindemuthianum*. **Pesquisa Agropecuaria Brasileira**, Brasília, DF, v. 44, n. 1, p. 15-21, jan. 2009.

CARUSO, C.; CHILOSI, G.; LEONARDI, L.; BERTINI, L.; MAGRO, P.; BUONOCORE, V.; CAPORALE, C. A basic peroxidase from wheat kernel with antifungal activity. **Phytochemistry**, Oxford, v. 58, n. 5, p. 743-750, Nov. 2001.

CAVALCANTI, F. R.; RESENDE, M. L. V.; CARVALHO, C. P.; SILVEIRA, J. A.; OLIVEIRA, J. T. An aqueous suspension of *Crinipellis perniciosa* mycelium activates tomato defence responses against *Xanthomonas vesicatoria*. **Crop Protection**, Oxford, v. 26, n. 5, p. 729-738, May 2007.

CAVALCANTI, F. R.; RESENDE, M. L. V.; LIMA, J. P. M. S.; SILVEIRA, J. A.; OLIVEIRA, J. T. Activities of antioxidant enzymes and photosynthetic responses in tomato pre-treated by plant activators and inoculated by *Xanthomonas vesicatoria*. **Physiological and Molecular Plant Pathology**, London, v. 68, n. 4/6, p. 198-208, Apr./June 2006.

CHOI, J. J.; ALKHAROUF, N. W.; SCHNEIDER, K. T. Expression patterns in soybean resistant to *Phakopsora pachyrhizi* reveal the importance of peroxidases and lipoxygenases. **Functional and Integrative Genomics**, Heidelberg, v. 8, n. 4, p. 341-359, Nov. 2008.

CONSTABEL, C. P.; BERGEY, D. R.; RYAN, C. Systemin activates synthesis of wound-inducible tomato leaf polyphenol oxidase via the octadecanoid defense signaling pathway. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, DC, v. 92, n. 2, p. 407-411, Jan. 1995.

CURTIS, M. D.; RAE, A. L.; RUSU, A. G.; HARRISON, S. J.; MANNERS, J. M. A peroxidase gene promoter induced by phytopathogens and methyl jasmonate in transgenic plants. **Molecular Plant-Microbe Interactions**, Saint Paul, v. 10, n. 3, p. 326-338, Apr. 1997.

DAMASCENO, C. M. B.; BISHOP, J. G.; RIPOLL, D. R.; WIN, J.; KARMOUN, S.; ROSE, J. K. C. Structure of the glucanase inhibitor protein (GIP) family from *Phytophthora* species suggests co-evolution with plant endo-beta-1,3-glucanases. **Molecular Plant-Microbe Interactions**, Saint Paul, v. 21, n. 6, p. 820-830, June 2008.

DANGL, J. L.; DIETRICH, R. A.; RICHBERG, M. H. Death don't have no mercy: cell death programs in plant-microbe interactions. **Plant Cell**, Rockville, v. 8, n. 10, p. 1793-1807, Oct. 1996.

DAW, B. D.; ZHANG, L. H.; WANG, Z. Z. Salicylic acid enhances antifungal resistance to *Magnaporthe grisea* in rice plants. **Australasian Plant Pathology**, Victoria, v. 37, n. 6, p. 637-644, Jan. 2008.

DEAN, B. B.; KOLATTUKUDY, P. E. Synthesis of suberin during woundhealing in jade leaves, tomato fruit, and bean pods. **Plant Physiology**, Rockville, v. 58, n. 3, p. 411-416, Jan. 1976.

DEMPSEY, D. A.; SHAH, J.; KLESSIG, D. F. Salicylic acid and disease resistance in plants. **Critical Reviews of Plant Sciences**, Boca Raton, v. 18, n. 4, p. 547-575, Jan. 1999.

DIXON, R. A.; HARRISON, M. J. Activation, structure, and organization of genes involved in microbial defense in plants. **Advances in Genetics**, New York, v. 28, n. 1, p. 165-234, Jan. 1990.

DURRANT, W. E.; DONG, X. Systemic acquired resistance. **Annual Review of Phytopathology**, Palo Alto, v. 42, n. 1, p. 185-209, Jan. 2004.

FARMER, E. E. Effects of fungal elicitor on lignin biosynthesis in cell suspension cultures of soybean. **Plant Physiology**, Rockville, v. 78, n. 2, p. 338-342, Jan. 1985.

FRICK, U. B.; SCHALLER, A. cDNA microarray analysis of fusicoccin-induced changes in gene expression in tomato plants. **Planta**, New York, v. 216, n. 1, p. 83-94, Nov. 2002.

FRY, S. C. Cross-linking of matrix polymers in the growing cell walls of angiosperms. **Annual Review of Plant Physiology**, Palo Alto, v. 37, p. 165-186, Jan. 1986.

FRY, S. C. Intracellular feruloylation of pectic polysaccharides. **Planta**, New York, v. 171, n. 2, p. 205-211, June 1987.

GIRLANDA, M.; BIANCIOTTO, V.; CAPPELLAZZO, G. A.; CASIERI, L.; BERGERO, R.; MARTINO, E.; LUPPI, A. M.; PEROTTO, S. Interactions between engineered tomato plants expressing antifungal enzymes and nontarget fungi in the rhizosphere and phyllosphere. **FEMS Microbiology Letters**, Oxford, v. 288, n. 1, p. 9-18, Nov. 2008.

GRAND, C.; SARNI, F.; LAMB, C. J. Rapid induction by fungal elicitor of the synthesis of cinnamyl-alcohol dehydrogenase, a specific enzyme of lignin synthesis. **European Journal of Biochemistry**, New York, v. 169, n. 1, p. 73-77, Nov. 1987.

GREENBERG, J. T. Programmed cell death in plant-pathogen interactions. **Annual Review of Plant Physiology and Plant Molecular Biology**, Palo Alto, v. 48, n. 1, p. 525-545, Jan. 1997.

GUEVARA, M. G.; OLIVA, C. R.; MACHINANDIARENA, M.; DALEO, G. R. Purification and properties of an aspartic protease from potato tuber that is inhibited by a basic chitinase. **Physiologia Plantarum**, Copenhagen, v. 106, n. 2, p. 164-169, June 1999.

GUO, Y.; XIONG, L.; SONG, C. P.; GONG, D.; HALFTER, U.; ZHU, J. K. A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signalling in Arabidopsis. **Development Cell**, Cambridge, v. 3, n. 2, p. 233-244, Aug. 2002.

HARRISON, S. J.; CURTIS, M. D.; MCINTYRE, C. L.; MACLEAN, D. J.; MANNERS, J. M. Differential expression of peroxidase isogenes during early stages of infection of the tropical forage legume *Stylosanthes humilis* by *Colletorichum gloeosporioides*. **Molecular Plant-Microbe Interactions**, Saint Paul, v. 8, n. 3, p. 398-406, May/June 1995.

HENRISSAT, B.; BAIROCH, A. Updating the sequence-based classification of glycosyl hydrolases. **Biochemical Journal**, London, v. 316, n. 2, p. 695-696, June 1996.

HERMAN, M. A. B.; RESTREPO, S.; SMART, C. D. Defense gene expression patterns of three SAR-induced tomato cultivars in the field. **Physiological and Molecular Plant Pathology**, Geneva, v. 71, n. 4/6, p. 192-200, Oct./Dec. 2007.

HILAIRE, E.; YOUNG, S. A.; WILLARD, L. H.; MCGEE, J. D.; SWEAT, T.; CHITTOR, J. M.; GUIKEMA, J. A.; LEACH, J. E. Vascular defense responses in rice: peroxidase accumulation in xylem parenchyma cells and xylem wall thickening. **Molecular Plant-Microbe Interactions**, Saint Paul, v. 14, n. 12, p. 1411-1419, Dec. 2001.

HIRAGA, S.; YAMAMOTO, K.; ITO, H.; SASAKI, K.; MATSUI, H.; HONMA, M.; NAGAMURA, Y.; SASAKI, T.; OHASHI, Y. Diverse expression profiles of 21 rice peroxidase genes. **FEBS Letters**, Amsterdam, v. 471, n. 2/3, p. 245-250, Apr. 2000.

HOEVEN, R. van der; RONNING, C.; GIOVANNONI, J.; MARTIN, G.; TANKSLEY, S. Deductions about the number, organization, and evolution of genes in the tomato genome based on analysis of a large expressed sequence tag collection and selective genomic sequencing. **Plant Cell**, Rockville, v. 14, n. 7, p. 1441-1456, July 2002.

HONÉE, G. Engineered resistance against fungal plant pathogens. **European Journal of Plant Pathology**, Dordrecht, v. 105, n. 4, p. 319-326, June 1999.
ISELI, B.; BOLLER, T.; NEUHAUS, J. M. The N-terminal cysteine-rich domain of tobacco class I chitinase is essential for chitin binding but not for catalytic or antifungal activity. **Plant Physiology**, Rockville, v. 103, n. 1, p. 221-226, Sept. 1993.

ISHIDA, A. K. N.; SOUZA, R. M.; ZACARONI, A. B.; RIBEIRO JÚNIOR, P. M.; AMARAL, D. R.; RESENDE, M. L. V. Extrato vegetal e acibenzolar-S-metil (ASM) na indução de resposta de defesa do algodoeiro contra *Xanthomonas axonopodis* pv. *malvacearum*. In: CONGRESSO BRASILEIRO DE FITOPATOLOGIA, 40., 2007, Maringá. **Fitopatologia Brasileira**, Lavras, v. 32, n. 6, p. S262, ago. 2007.

JIANG, F.; ZHENG, X.; CHEN, J. Microarray analysis of gene expression profile induced by the biocontrol yeast *Cryptococcus laurentii* in cherry tomato fruit. **Gene**, Amsterdam, v. 430, n. 1/2, p. 12-16, Feb. 2009.

JIN, P.; ZHENG, Y. H.; TANG, S. S.; RUI, H. J.; WANG, C. Y. Enhancing disease resistance in peach fruit with methyl jasmonate. **Journal of the Science of Food and Agriculture**, Sussex, v. 89, n. 5, p. 802-808, Mar. 2009.

JOSHI, B. N.; SAINANI, M. N.; BASTAWADE, K. B.; GUPTA, V. S.; RANJEKAR, P. K. Cysteine protease inhibitor from pearl millet: a new class of antifungal protein. **Biochemical and Biophysical Research Communications**, San Diego, v. 246, n. 2, p. 382-387, May 1998.

JWANNY, E. W.; EL-SAYED, S.; SALEM, A. M.; SHEHATA, A. N. Characterization and antifungal evaluation of chitinase and laminarinases from sugar beet leaves. **Pakistan Journal of Biology Science**, Pakistan, v. 4, n. 1, p. 271-276, Jan. 2001.

KANG, N. J. Induced resistance to powdery mildew by 2,6-dichloroisonicotinic acid is associated with activation of active oxygen species-mediated enzymes in cucumber plants. **Journal of the Japanese Society for Horticultural Science**, Kyoto, v. 78, n. 2, p. 185-194, Apr. 2009.

KAVITHA, R.; UMESHA, S. Regulation of defense-related enzymes associated with bacterial spot resistance in tomato. **Phytoparasitica**, Rehovot, v. 36, n. 2, p. 144-159, Mar. 2008.

KAWAOKA, A.; MATSUNAGA, E.; ENDO, S.; KONDO, S.; YOSHIDA, K.; SHINMYO, A.; EBINUMA, H. Ectopic expression of a horseradish peroxidase enhances growth rate and increases oxidative stress resistance in hybrid aspen. **Plant Physiology**, Rockville, v. 132, n. 3, p. 1177-1185, July 2003.

KIM, J. S.; SAGARAM, U. S.; BURNS, J. K.; LI, J. L.; WANG, N. Response of sweet orange (*Citrus sinensis*) to 'candidatus liberibacter asiaticus' infection: microscopy and microarray analyses. **Phytopathology**, Saint Paul, v. 99, n. 1, p. 50-57, Jan. 2009.

KRISTENSEN, B. K.; BLOCH, H.; RASMUSSEN, S. K. Barley coleoptile peroxidases: purification, molecular cloning, and induction by pathogens. **Plant Physiology**, Rockville, v. 120, n. 2, p. 501-512, June 1999.

KUC, J. Concepts and direction of induced systemic resistance in plants and its application. **European Journal of Plant Pathology**, Dordrecht, v. 107, n. 1, p. 7-12, Jan. 2001.

LAGRIMINI, L. M.; GINGAS, V.; FINGER, F.; ROTHSTEIN, S.; LIU, T. Characterization of antisense transformed plants deficient in the tobacco anionic peroxidase. **Plant Physiology**, Rockville, v. 114, n. 4, p. 1187-1196, Aug. 1997.

LAGRIMINI, L. M.; ROTHSTEIN, S. Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. **Plant Physiology**, Rockville, v. 84, n. 2, p. 438-442, June 1987.

LAMB, C.; DIXON, R. A. The oxidative burst in plant disease resistance. **Annual Review of Plant Physiology and Plant Molecular Biology**, Palo Alto, v. 48, n. 1, p. 251-275, Jan. 1997.

- LI, L.; STEFFENS, J. C. Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. **Planta**, New York, v. 215, n. 2, p. 239-247, June 2002.
- LOON, L. C. van; PIERPOINT, W. S.; BOLLER, T.; CONEJERO, V. Recommendations for naming plant pathogenesis-related proteins. **Plant Molecular and Biology Reporter**, Athens, v. 12, n. 1, p. 245-264, Jan. 1994.
- LUTZ, M.; FEICHTINGER, G.; DEFAGO, G.; DUFFY, B. Mycotoxigenic *Fusarium* and deoxynivalenol production repress chitinase gene expression in the biocontrol agent *Trichoderma atroviride* P1. **Applied and Environmental Microbiology**, Washington, DC, v. 69, n. 6, p. 3077-3084, June 2003.
- MAJEAU, N.; TRUDEL, J.; ASSELIN, A. Diversity of cucumber chitinase isoforms and characterization of one seed basic chitinase with lysozyme activity. **Plant Science**, Clare, v. 68, n. 1, p. 9-16, Jan. 1990.
- MALECK, K.; LEVINE, A.; EULGEM, T.; MORGAN, A.; SCHMID, J.; LAWTON, K. A.; DANGL, J. L.; DIETRICH, R. A. The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. **Nature Genetics**, New York, v. 26, n. 4, p. 403-410, Dec. 2000.
- MAUCH, F.; MAUCH-MANI, B.; BOLLER, T. Antifungal hydrolases in pea tissue: II., inhibition of fungal growth by combinations of chitinase and beta-1,3-glucanase. **Plant Physiology**, Rockville, v. 88, n. 3, p. 936-942, Nov. 1988.
- MAYER, A. M. Polyphenol oxidases in plants: recent progress. **Phytochemistry**, Oxford, v. 26, n. 1, p. 11-20, Jan. 1987.
- MAYER, A. M.; HAREL, E. Polyphenol oxidase in plants. **Phytochemistry**, Oxford, v. 18, n. 1, p. 193-215, Jan. 1979.
- MELCHERS, L. S.; APOTHEKER-DE-GROOT, M.; KNAAP, J. A. van der; PONSTEIN, A. S.; SELA-BUURLAGE, M. B.; BOL, J. F.; CORNELISSEN, B. J.; ELZEN, P. J. van den; LINTHORST, H. J. A new class of tobacco chitinases homologous to bacterial exo-chitinases displays antifungal activity. **Plant Journal**, Oxon, v. 5, n. 4, p. 469-480, Apr. 1994.
- MERCIER, L.; LAFITTE, C.; BORDERIES, G.; BRIAND, X.; ESQUERRÉ-TUGAYÉ, M. T.; FOURNIER, J. The algal polysaccharide carrageenans can act as an elicitor of plant defence. **New Phytologist**, New York, v. 149, n. 1, p. 43-51, Jan. 2001.

MÉTRAUX, J. P. Systemic acquired resistance and salicylic acid: current state of knowledge. **European Journal of Plant Pathology**, Dordrecht, v. 107, n. 1, p. 13-18, Jan. 2001.

MONTESANO, M.; BRADER, G.; PALVA, E. T. Pathogen derived elicitors: searching for receptors in plants. **Molecular Plant Pathology**, Oxon, v. 4, n. 1, p. 73-79, Jan. 2003.

MOORE, S.; PAYTON, P.; WRIGHT, P.; TANKSLEY, S.; GIOVANNONI, J. Utilization of tomato microarrays for comparative gene expression analysis in the Solanaceae. **Journal of Experimental Botany**, Oxford, v. 56, n. 421, p. 2885-2895, Nov. 2005.

NEUHAUS, J. M.; FRITIG, B.; LINTHORST, H. J. M.; MEINS, F.; MIKKELSEN, J. D.; RYALS, J. A revised nomenclature for chitinase genes. **Plant Molecular Biology Report**, New Brunswick, v. 14, n. 2, p. 102-104, June 1996.

NICHOLSON, R. L.; HAMMERSCHMIDT, R. Phenolic compounds and their role in disease resistance. **Annual Review of Phytopathology**, Palo Alto, v. 30, n. 1, p. 369-389, Jan. 1992.

PELTIER, A. J.; HATFIELD, R. D.; GRAU, C. R. Soybean stem lignin concentration relates to resistance to *Sclerotinia sclerotiorum*. **Plant Disease**, Saint Paul, v. 93, n. 2, p. 149-154, Feb. 2009.

PEUMANS, W. J.; PROOST, P.; SWENNEN, R. L.; DAMME, E. J. van. The abundant class III chitinase homolog in young developing banana fruits behaves as a transient vegetative storage protein and most probably serves as an important supply of amino acids for the synthesis of ripening-associated proteins. **Plant Physiology**, Rockville, v. 130, n. 2, p. 1063-1072, Oct. 2002.

QUIROGA, M.; GUERRERO, C.; BOTELLA, M. A.; BARCELÓ, A.; AMAYA, I.; MEDINA, M. I.; ALONSO, F. J.; FORCHETTI, S. M. de; TIGIER, H.; VALPUESTA, V. A tomato peroxidase involved in the synthesis of lignin and suberin. **Plant Physiology**, Rockville, v. 122, n. 4, p. 1119-1127, Apr. 2000.

REIMERS, P. J.; LEACH, J. E. Race-specific resistance to *Xanthomonas oryzae* pv. *oryzae* conferred by bacterial blight resistance gene Xa-10 in rice (*Oryza sativa*) involves accumulation of a lignin-like substance in host tissues. **Physiological and Molecular Plant Pathology**, London, v. 38, n. 1, p. 39-55, Jan. 1991.

RESENDE, M. L. V.; ARAUJO, D. V.; COSTA, J. C. B.; DEUNER, C. C.; FERREIRA, J. B.; MUNIZ, M. F. S.; REIS, S. N.; SANTOS, F. S.; CAVALCANTI, L. S.; NOJOSA, G. B. A. Produtos comerciais a base de bioindutores de resistência em plantas. **Revisão Anual de Patologia de Plantas**, Passo Fundo, v. 14, n. 1, p. 361-380, Jan. 2006.

RIDE, J. P. The role of cell wall alterations in resistance to fungi. **Annals of Applied Biology**, Warwick, v. 89, n. 1, p. 302-306, Jan. 1978.

RIDE, J. P.; PEARCE, R. B. Lignification and papilla formation at sites of attempted penetration of wheat leaves by non-pathogenic fungi. **Physiological Plant Pathology**, London, v. 15, n. 1, p. 79-92, Jan. 1979.

ROBERTSA, P. D.; MOMOLB, M. T.; RITCHIEB, L.; OLSONB, S. M.; JONESC, J. B.; BALOGH, B. Evaluation of spray programs containing famoxadone plus cymoxanil, acibenzolar-S-methyl, and *Bacillus subtilis* compared to copper sprays for management of bacterial spot on tomato. **Crop Protection**, Oxford, v. 27, n. 12, p. 1519-1526, Dec. 2008.

SANTOS, F. S.; SOUZA, P. E.; RESENDE, M. L. V.; POZZA, E. A.; MIRANDA, J. C.; RIBEIRO JÚNIOR, P. M.; MANERBA, F. C. Efeito de extratos vegetais no progresso de doenças foliares do cafeeiro orgânico. **Fitopatologia Brasileira**, Lavras, v. 32, n. 1, p. 59-63, fev. 2007.

SHARP, J. K.; MCNEIL, M.; ALBERSHEIM, P. The primary structures of one elicitor-active and seven elicitor-inactive hexa ((3-D glucopyranosyl)-D-glucitols isolated from the mycelial walls of *Phytophthora megasperma* f. sp. *glycinea*. **Journal of Biological Chemistry**, Bethesda, v. 259, n. 18, p. 321-336, Jan. 1984.

SHOWALTER, A. M. Structure and function of plant cell wall proteins. **Plant Cell**, Rockville, v. 5, n. 1, p. 9-23, Jan. 1993.

STASKAWICZ, B. J.; AUSUBEL, F. M.; BAKER, B. J.; ELLIS, J. G.; JONES, J. D. G. Molecular genetics of plant disease resistance. **Science**, Washington, DC, v. 268, n. 5211, p. 661-667, May 1995.

STEFFENS, J. C.; HAREL, E.; HUNT, M. D. Polyphenol oxidase. In: ELLIS, B. E.; KUROKI, G. W.; STAFFORD, H. A. (Ed.). **Genetic engineering of plant secondary metabolism**. New York: Plenum, 1994. p. 275-312.

STINTZI, A.; HEITZ, T.; PRASAD, V.; WIEDEMANN-MERDINOGLU, S.; KAUFFMANN, S.; GEOFFROY, P.; LEGRAND, M.; FRITIG, B. Plant 'pathogenesis-related' proteins and their role in defense against pathogens. **Biochimie**, Paris, v. 75, n. 8, p. 687-706, Jan. 1993.

STOUT, M. J.; FIDANTSEF, A. L.; DUFFEY, S. S.; BOSTOCK, R. M. Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. **Physiological and Molecular Plant Pathology**, London, v. 54, n. 3/4, p. 115-130, Apr./May 1999.

TABAEIZADEH, Z.; AGHARBAOUI, Z.; HARRAK, H.; POYSA, V. Transgenic tomato plants expressing a *Lycopersicon chilense* chitinase gene demonstrate improved resistance to *Verticillium dahliae* race 2. **Plant Cell Reports**, New York, v. 19, n. 2, p. 197-202, Dec. 1999.

THALER, J. S.; FIDANTSEF, A. L.; DUFFEY, S. S.; BOSTOCK, R. M. Trade-offs in plant defense against pathogens and herbivores: a field demonstration of chemical elicitors of induced resistance. **Journal of Chemical Ecology**, New York, v. 25, n. 7, p. 1597-609, July 1999.

THEIS, T.; STAHL, U. Antifungal proteins: targets, mechanisms and prospective applications. **Cellular and Molecular Life Sciences**, Basel, v. 61, n. 4, p. 437-455, Feb. 2004.

THIPYAPONG, P.; HUNT, M. D.; STEFFENS, J. C. Systemic wound induction of potato (*Solanum tuberosum*) polyphenol oxidase. **Phytochemistry**, Oxford, v. 40, n. 3, p. 673-676, Oct. 1995.

THIPYAPONG, P.; JOEL, D. M.; STEFFENS, J. C. Differential expression and turnover of the tomato polyphenol oxidase gene family during vegetative and reproductive development. **Plant Physiology**, Rockville, v. 113, n. 3, p. 707-718, Mar. 1997.

THIPYAPONG, P.; STEFFENS, J. C. Tomato polyphenol oxidase (PPO): differential response of the PPO F promoter to injuries and wound signals. **Plant Physiology**, Rockville, v. 115, n. 2, p. 409-418, Oct. 1997.

THORDAL-CHRISTENSEN, H.; BRANDT, J.; CHO, B. H.; RASMUSSEN, S. K.; GREGERSEN, P. L.; SEMEDEGAARD-PETERSEN, V.; COLLINGE, D. B. cDNA cloning and characterization of two barley peroxidase transcripts induced differentially by the powdery mildew fungus *Erysiphe graminis*. **Physiological and Molecular Plant Pathology**, London, v. 40, n. 1, p. 395-409, Jan. 1992.

UNIVERSIDADE FEDERAL DE LAVRAS. RESENDE, M. L. V.; CAVALCANTI, F. R.; SANTOS, F. S.; RIBEIRO JUNIOR, P. M.; AMARAL, D. R. **Formulação para indução de resistência em plantas, a base de extrato vegetal obtido de folhas do cafeeiro**. BR n. INPI 0000220604167501. 2 ago. 2006.

VALLAD, G. E.; GOODMAN, R. M. Systemic acquired resistance and induced systemic resistance in conventional agriculture. **Crop Science**, Madison, v. 44, n. 6, p. 1920-1934, Nov./Dec. 2004.

VANCE, C. P.; KIRK, T. K.; SHERWOOD, R. T. Lignification as a mechanism of disease resistance. **Annual Review of Phytopathology**, Palo Alto, v. 18, n. 1, p. 259-288, Jan. 1980.

VERA, P.; TORNERO, P.; CONEJERO, V. Cloning and expression analysis of a viroid-induced peroxidase from tomato plants. **Molecular Plant-Microbe Interaction**, Saint Paul, v. 6, n. 6, p. 790-794, Nov./Dec. 1993.

YEH, S.; MOFFATT, B. A.; GRIFFITH, M.; XIONG, F.; YANG, D. S.; WISEMAN, S. B.; SARHAN, F.; DANYLUK, J.; XUE, Y. Q.; HEW, C. L.; DOHERTY-KIRBY, A.; LAJOIE, G. Chitinase genes responsive to cold encode antifreeze proteins in winter cereals. **Plant Physiology**, Rockville, v. 124, n. 3, p. 1251-1264, Nov. 2000.

YOUNG, S. A.; GUO, A.; GUIKEMA, J. A.; WHITE, F. F.; LEACH, J. E. Rice cationic peroxidase accumulates in xylem vessels during incompatible interactions with *Xanthomonas oryzae* pv. *oryzae*. **Plant Physiology**, Rockville, v. 107, n. 4, p. 1333-1341, Apr. 1995.

CHAPTER 2

Induction of pathogenesis-related proteins and resistance by a natural formulation of coffea leaf and acibenzolar-S-methyl in tomato seedlings against bacterial spot.

ABSTRACT

The efficiency of a natural formulation of coffee leaf (NEFID) and the plant defense inducing compound, acibenzolar S-methyl (ASM) was tested, on tomato seedling growth and on induced defense response against bacterial spot. Plantlets were sprayed with either NEFID or ASM and subsequently challenged with a virulent strain of *Xanthomonas vesicatoria* 3 days after. Disease severity, plant growth and leaf area were evaluated. To understand defense related responses, PR-1 and β -1,3-glucanase gene expression patterns were evaluated as well as the induction of β -1,3-glucanase (GLU), chitinase (CHI), polyphenol oxidase (PPO) activity, and lignin deposition, were compared in treated and control plants. NEFID and ASM treated plants demonstrated a reduced disease severity by 35 and 61% respectively. This reduction in disease severity was correlated with increases in β -1,3-glucanase and PR-1 gene expression 12 hours after spraying and elevated chitinase, glucanase and polyphenol oxidase activity 24 hours after spraying; higher leaf lignin deposition was also observed at 10 and 23 days after spraying.

Keywords: systemic acquired resistance, coffea natural formulation, PR-1, chitinase, glucanase, polyphenol oxidase, lignin

RESUMO

A eficiência de uma formulação natural a base de folha de café (NEFID) e do composto comercial indutor de resistência em plantas acibenzolar S-metil (ASM) foi testada no crescimento de mudas de tomateiro e na resposta de defesa induzida contra a mancha bacteriana. Plântulas foram pulverizadas com NEFID ou ASM e subsequentemente inoculadas com uma estirpe virulenta de *Xanthomonas vesicatoria*, três dias após o tratamento. Foram avaliados a severidade da doença, o crescimento da planta e a área foliar. Para melhor entender os mecanismos de resposta de defesa da planta, padrões de expressão dos genes PR-1 e β -1,3-glucanase foram também avaliados assim como, a atividade das enzimas β -1,3-glucanase (GLU), quitinase (CHI), polifenol oxidase (PPO) e deposição de lignina foram comparados em plantas tratadas e controle. Plantas pulverizadas com NEFID e ASM demonstraram redução da severidade da doença em 35 e 61% respectivamente. Esta redução na severidade da doença foi correlacionada com o aumento na expressão dos genes β -1,3-glucanase e PR-1, 12 horas após a pulverização e, a uma elevada atividade de quitinase, glucanase e polifenol oxidase 24 horas após a pulverização; uma maior deposição de lignina nas folhas foi também observada aos 10 e 23 dias após a pulverização.

Palavras-chave: Resistência sistêmica adquirida, formulação natural a base de café, PR-1, quitinase, glucanase, polifenol oxidase, lignina

1 INTRODUCTION

The bacterial spot (*Xanthomonas vesicatoria* Doidge) is a serious seed-borne tomato disease efficiently seed-transmitted. Carmo et al. (1996) showed that only one seed out of 10,000 in a seed-lot is a sufficient inoculum for a 100% bacterial spot incidence in bell pepper under favorable environmental conditions, such as the one that prevails under tropical growing regions (Al-Dahmani et al., 2003).

While seed testing does not eliminate the possible seed-lot contamination and efficient seed treatment still has detrimental consequences on germination (Carmo et al., 2004), plant protection strategies can improve the health and productivity of tomato plants

The benzothiadiazole derivative benzo(1,2,3)thiazole-7-carbothioic acid-S-methyl ester (acibenzolar-S-methyl, ASM, or BTH) has been developed as a potent systemic acquired resistance (SAR) activator that does not have antimicrobial properties, but instead increases crop resistance to disease by activating the SAR signal transduction pathway in several plant species including tomato (Achoo et al., 2004; Bokshi et al., 2003; Lawton et al., 1996; Soyly et al., 2003). The use of acibenzolar-S-methyl protected plants by 47.7% compared to the water pre-treated control and the well-known mechanism is the salicylic acid resistance (SAR) induction by the activation of defense-related enzymes peroxidase and polyphenol oxidase starting within hours after spraying (Cavalcanti et al., 2006).

The SAR is also associated with the induction of chitinases, β -1,3-glucanases and other pathogenesis-related (PR) proteins that can hamper not only the development of bacterial pathogens but also fungal, nematodal and even viral-associated plant diseases (Van Loon & Van Strien, 1999) but also make it difficult for pathogens to develop resistance (Hammond-Kosack &

Parker, 2003). Induction of plant defenses against pathogens also involves physical barriers such as cell wall reinforcement via lignin deposition (Anterola & Lewis, 2002; Thangavelu et al., 2003) resulting of an increase in enzyme activities, such as phenylalanine ammonia-lyase and polyphenol oxidases, related to the phenylpropanoid pathway (He et al., 2002). Similar activation of SAR is observed after exposition of plants to microbial surface-derived molecules such as peptides, carbohydrates, glycoproteins and lipids or plant derived cell wall (Nürnberg & Brunner, 2002). An infected coffee leaf formulation EFID combines both fungal and plant derived elicitors and has efficiently controlled coffee rust under field conditions and induced the accumulation of lignin compared to the water pre-treated control (Santos et al., 2007).

The aim of this research was to compare the effectiveness of a natural formulation of coffee leaf NEFID and that of a known plant defense inducing compound, – ASM, on tomato seedling growth and on resistance induction against *X. vesicatoria* inoculation. The present study was also aimed at understanding the defense related response by comparing the expression pattern of selected defense related mRNAs and the induction of the corresponding proteins after treatment with chemical and biological elicitors.

2 MATERIAL AND METHODS

2.1 Plant material and inoculum preparation

For the biochemical determinations and the disease severity measurement tomato seeds (*Solanum lycopersicum* var. Santa Cruz Kada) susceptible to *X. vesicatoria*, purchased from Isla Sementes Ltda (Porto Alegre — Rio Grande do Sul, Brazil) were surface sterilized in 1% (v/v) ethanol for 3 min, followed by 1.0 g L⁻¹ sodium hypochlorite for 1 min. After thorough rinsing with distilled water they were planted in 3 L pots filled with Plantmax® substrate in a greenhouse at a mean temperature cycle of 28±3°C day/23±3°C night, relative humidity of 40±3%/85±3%, and a 12 h photoperiod, at 450–500 mmolm⁻² s⁻¹ maximum photon flux densities, measured at plant level (IRGA, model LCA-4, Hoddesdon, UK). For analysis by reverse transcription–polymerase chain reaction (RT–PCR) experiments were conducted in a growth chamber in which temperature was maintained at 29±4°C with a relative humidity 40±10%. Plants were grown under metal halide and high pressure sodium lamps for a 16-h/8-h light/dark photoperiod with a total light intensity of 700 mmol/m²/s.

Twenty-five days after planting, when seedlings were 20±4 cm tall, the tomato leaves were sprayed with the test substances in order to determine their capacity to protect themselves from bacterial spot.

Bacterial cultures of *Xanthomonas vesicatoria* strain 89-T from the Embrapa's (Centro Nacional de Pesquisa de Hortaliças) culture collection were cultivated at 28°C in a culture media Kado & Heskett 523. Inoculum was obtained from bacterial cells during the log-phase growth period, cultured in a liquid Kado & Heskett 523 medium and incubated under shaking at 200 rpm at 28°C for 12 h (dark). The bacterial cells were concentrated by centrifugation (twice each at 3000g, 5 min) and resuspended in sterile distilled water.

Inoculum concentration was adjusted by dilution with sterile distilled water to give an absorbance of 0.200 at 540 nm, corresponding to 10^{11} cfu L⁻¹. For long-term storage, bacterial cultures were maintained at -80°C in a liquid Kado & Heskett 523 medium that contained 20% (v/v) glycerol.

2.2 NEFID and ASM

NEFID formulation which has as its main raw material coffee leaves (*Coffea arabica*), collected from field soil surface (due to disease, harvest fruit and/or other stresses) and selected for powder production. A hundred grams of this powder is mixed with 1000 mL distilled water, boiled in reflux and filtered through a sieve of 400 meshes. The filtrate of leaves is sampled and stored at -20°C. The formulation based on NEFID is patented by Resende et al. (2006) **(I.N.P.I., Protocolo número 0000220604167501, FORMULAÇÃO PARA INDUÇÃO DE RESISTÊNCIA... 02 de Agosto de 2006).**

Bion® [acibenzolar-S-methyl or benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester] (ASM) purchased from Syngenta Proteção de Cultivos Ltda in the form of a 500 g kg⁻¹ dry powder active ingredient (ASM) and used as a 0.2 g ASM L⁻¹ aqueous solution.

2.3 Treatment, assessment of disease severity and preparation of samples for determining gene expression and enzyme activities.

Intended for disease severity, tomato plants at 25 days after planting were uniformly sprayed on the aerial portion of the plant with ASM, NEFID or distilled water (control) until runoff. Three days after treatment the bacterial inoculation was performed, spraying 80 mL of *Xanthomonas vesicatoria* cell suspension (10^{11} cfu per L⁻¹) until runoff, in control and treated inoculated tomato plants. Disease symptoms were evaluated at seven days after bacterial inoculation. The severity of bacterial spot was assessed by visual diagrammatic scale rating of lesions on a 1-50% (the percentage of lesions present on the total

leaf area) (Mello et al., 1997). Leaf area was evaluated at the end of the trial period 20 days after spraying, using ImageJ software. Seedlings heights were evaluated at 3, 10, 13, 16 and 20 days after spraying.

Designed for analysis by reverse transcription–polymerase chain reaction (RT–PCR), plants were harvested at different time points (12, 24, 48, 72, 96, 144 h) after the treatment spraying. All plant materials were harvested during the light period at 1:00 p.m. to avoid light/dark effects on gene expression. Individual plants were used for each time-point harvest.

For enzymatic assays, 25 day-old tomato plants were sprayed with ASM, NEFID or distilled water (control) until runoff. Treated and control plants were harvested at 0, 24, 48, 72, 96, 120 and 240 hours after sprayings (HAS). In an additional trial, 25 day-old plants were sprayed with the test substances and three days later challenged by inoculation with 80 mL of *X. vesicatoria* cell suspension (10^{11} cfu l⁻¹). The excised leaves were frozen in liquid nitrogen and immediately utilized.

2.4 Reverse transcriptase-mediated PCR

Total leaf RNA was isolated with an RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Five micrograms of RNA was reverse-transcribed into cDNA using 2µL MuMLV-RT enzyme (Promega, Madison, WI, USA). Each reaction mixture contained 5 µg of RNA, 4µL oligo dT, 4 µL dNTPs (2.5 M), 1µL RNasin, 6µL DEPC water and 10µL of 5X MuMLV buffer, and samples were incubated at 37° C for 1 h. For the determination of transcript quantities, the first strand cDNA was amplified in a PCR reaction using gene-specific primers:

- *pr-1*: 5'-ACTCAAGTAGTCTGGCGCAACTCA-3' and 5'-AGTAAGGACGTTGTCCGATC CAGT-3' - *Lycopersicon esculentum*

pathogenesis-related protein (PR1) mRNA gi|76363946| gb|DQ159948.1| with a product size of 124bp.

- *glu*: 5'-AAGCAATCGGTGAAGCTGGTTTGG-3' and 5'-ATGGCCATCCACTCTCTGAC ACAA-3', *Lycopersicon esculentum* beta-1,3-glucanase mRNA, gi|170381|gb|M80608.1| TOMB13GLUB, with a product size of 381bp.

- *act*: 5'-TTGACTGAGGCACCACTTAACCCT-3' and 5'-GCTTTCAGGTGGTGCAACGACTTT-3', *Lycopersicon esculentum* actin gene, gi|1498360|gb|U60478.1|SLU60478, with a product size of 777bp.

The PCR volume was 50µl, containing 100 ng of each primer, 4µl dNTPs, 5µl of cDNA, and 0.5 units of Taq DNA polymerase (Fisher Scientific, USA). A Programmable Thermal Controller (MJ Research, Watertown, MA, USA) was used for the amplification of *pr-1*, *glu*, *act* cDNAs, using 25 cycles, under optimum dynamic ranges before reaching the plateau. Equal amounts of PCR products were separated by electrophoresis in 1.5% agarose gels and detected by staining with ethidium bromide. *Lycopersicon esculentum* actin, was used to ensure equal loading of the lanes. RT-PCR analysis was performed at least in triplicate. For both experiments, agarose gel electrophoresis images were taken by Kodak Gel Logic 100 Imaging System (Fisher Scientific, Houston, TX, USA) and the band intensity quantified by Image J 1.33u (<http://rsb.info.nih.gov/ij/>, National Institute of Health, USA). Normalization of signal was based on the housekeeping gene *Solanum lycopersicum* actin (NCBI gi|1498360).

2.5 Enzyme extraction, PR-protein, defense enzyme assay

Treated and control, non-inoculated collected at 0, 24, 48, 72, 96, 144 and 240 hours after spraying (HAS) and inoculated with *X. vesicatoria* assemble at 72, 96, 144 and 240 HAS, tomato fresh leaf (1.0 g) was

homogenized for 5 min in a mortar with a pestle in 3 mL of ice-cold 50mM sodium acetate buffer pH 5.2, containing 0.1mM EDTA. After filtration, the homogenate was centrifuged at 13,000g for 15 min and the supernatant (crude extract) used as the source of enzymes. All the steps were carried out at 0–4° C (Cavalcanti et al., 2007). Protein content of the crude extracts was determined using the Bradford (1976) protein assay, with bovine serum albumin (BSA) as a standard.

Chitinase activity CHI (PR-3; EC 3.2.1.14) was determined by adding 70 μL of suitably diluted crude extract with 130 μL of 50 mM sodium acetate pH 5.2 and 60 μL of CM-Chitin-RBV (2 mg mL^{-1}), a polymeric carboxymethyl-substituted chitin, labelled covalently with Remazol Brilliant Violet 5R (CM-Chitin-RBV, Loewe, Biochemica, Germany) used as substrate, in microplates of 96 wells with a capacity of 350 μL . After incubation at 35° C for 80 min, samples were acidified with 50 μL of 0.5 N HCl, cooled in ice bath for 10 min and centrifuged (1,450 g for 10 min). Absorbance of the supernatant at 492nm was recorded and the results were expressed as UA. One unit of CHI activity was defined as the variation of one absorbance unit at 492 nm per milligram of soluble protein per minute ($\text{UA mg}^{-1} \text{min}^{-1}$). Assays were carried out in triplicate.

The activity of beta-1,3-glucanase GLU (PR-2; EC 3.2.1.39) was measured using similar method, with the exchange of the substrate for CM-Curdlan-RBB (4mg mL^{-1}) and the adjustment of the rate of enzyme extract to 100 μL (minus the volume of acetate buffer in order to adjust the final volume to 310 μL per cavity). To promote the hydrolytic action of beta-1 3-glucanase was adopted incubation period of 35° C for 100 min. Absorbance of the supernatant at 620 nm was recorded and the results were expressed as UA. One unit of CHI activity was defined as the variation of one absorbance unit at 620

nm per milligram of soluble protein per minute (UA mgP⁻¹ min⁻¹). Assays were carried out in triplicate.

The activity of PPO (EC 1.10.3.2) was determined by adding 50 μ L of the crude extract to 3 mL of a solution containing 100mM potassium phosphate buffer, pH 6.5 and 25mM pyrocatechol. The increase of absorbance at 410 nm, for 10 min at 30° C, was measured (Gauillard et al., 1993). One PPO unit was expressed as the variation of absorbance at 410 nm per milligram of soluble protein per minute (UA mgP⁻¹ min⁻¹).

For lignin quantification, the assay described by Monties (1989) was used with minor modifications (Cavalcanti et al., 2006). Samples of 0.2 mg dry leaf tissue were powdered with liquid nitrogen and incubated with 85% acetone for 48 h. After centrifugation at 7500 g for 15 min at 4°C, the green supernatant was discarded and the remaining ketonic precipitate was air-dried, re-suspended in 5 mL thioglycolic acid (TGA) (SIGMA) prepared in 2N HCl (1:10, v/v) and left for 4 h at 25°C. Next it was centrifuged at 7500 g for 15 min at 4°C and the resulting supernatant transferred to a fresh 20 mL tube to which 200 mL 10M HCl was added. After incubation in an ice-bath for 4 h, this mix was centrifuged (7500g, 30 min, 7°C), the pellet was homogenized in 5 mL 0.5N NaOH, and the absorbance at 280nm was measured. TGA derivatives (acid-soluble lignin) formed were quantified by comparison with a standard curve prepared with known amounts (10–100 mg mL⁻¹) of 2-hydroxypropyl ether. Assays were done in triplicate and data expressed as micrograms per milligrams of dry weight.

2.6 Experimental design and statistical methods

The disease severity was measured in greenhouse conditions, experiments (twice) were arranged in randomized block designs with four blocks, and one experimental unit (plot) consisted of four 3 L pot containing

two plants. For the biochemical determinations, experiments were arranged in randomized block designs with three blocks, in a factorial scheme and one experimental unit (plot) consisted of four 3 L pot containing three plants each.

For analysis by reverse transcription–polymerase chain reaction (RT–PCR) experiments plants were arranged in randomized blocks designs with three blocks, and one experimental unit (plot) consisted of four 1.5 L pot containing two plants each.

Variance analysis was run using SAS (Statistical Analysis Systems Inc., Cary, NC, USA) statistical software. Means were separated using Tukey's test at P value less than 0.05 using Sisvar, a statistical tool purchased from the Federal University of Lavras, Minas Gerais, Brazil.

3 RESULTS

3.1 Effects of foliar sprays of NEFID and ASM on the disease severity of bacterial spot and plant development under greenhouse conditions.

First symptoms of bacterial spot of tomato inoculated with *X. vesicatoria* were detected 72 hours after inoculation. A coffee leaf formulation has shown to control tomato bacterial spot and this response occurred as early as 7 days after inoculation with 35% disease severity reduction, a significantly reduction (61%) was obtained by the commercial plant defense activator Actiguard (Acibenzolar-S-methyl) (Figure 1).

The plant growth was measured at 3, 7, 10, 13, 16 and 20 days after spraying. Plants treated with the disease resistance elicitors had previously shown to have a larger canopy than water pre-treated controls. By measuring plant height over time and analyzing the overall growth both elicitors, coffee leaf formulation – NEFID and acibenzolar-S-methyl, improved plant growth (9.68 and 13.56%, respectively) at 13 DAS (Table 1), after this time, only plants sprayed with ASM had shown enhanced in the seedlings growth compared with water pre-treated plants. The commercial elicitor also improved leaf area (93%) while NEFID did not affect leaf growth (Figure 2). The leaf area was measured at the end of the experiment, 20 days after spraying.

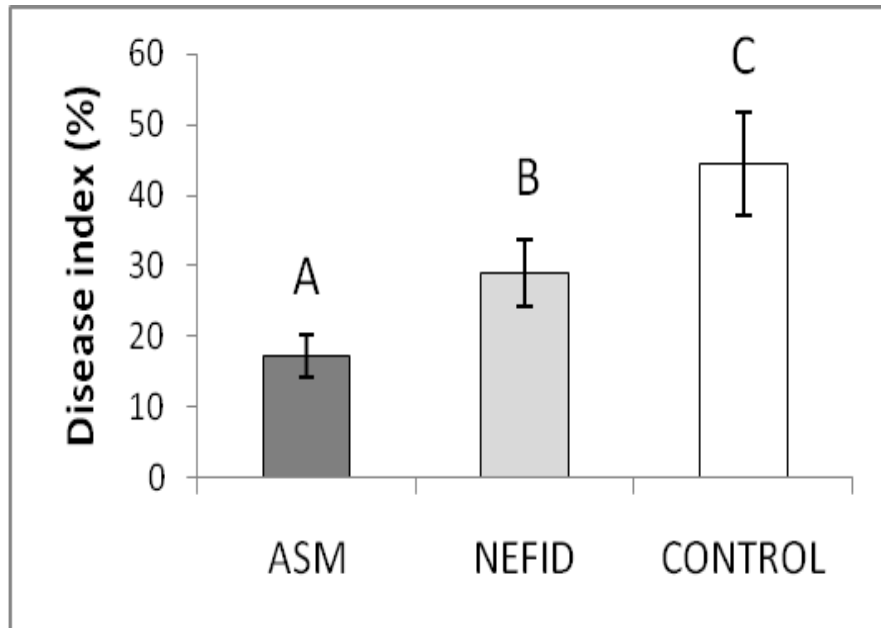


FIGURE 1 Coffee leaves formulation (NEFID) and the commercial disease resistance inducer ASM (Acibenzolar-S-methyl) reduced the severity of tomato bacterial spot caused by *X. vesicatoria* in a susceptible cultivar, 'St. Cruz Kada' compared to the water pre-treated seedlings (control) seven days after inoculation. Tested substances were sprayed on leaves 25 days after planting and plants were inoculated 3 days later. Bars followed by the same letter are not significantly different according to Tukey's multiple range test ($P \leq 0.05$).

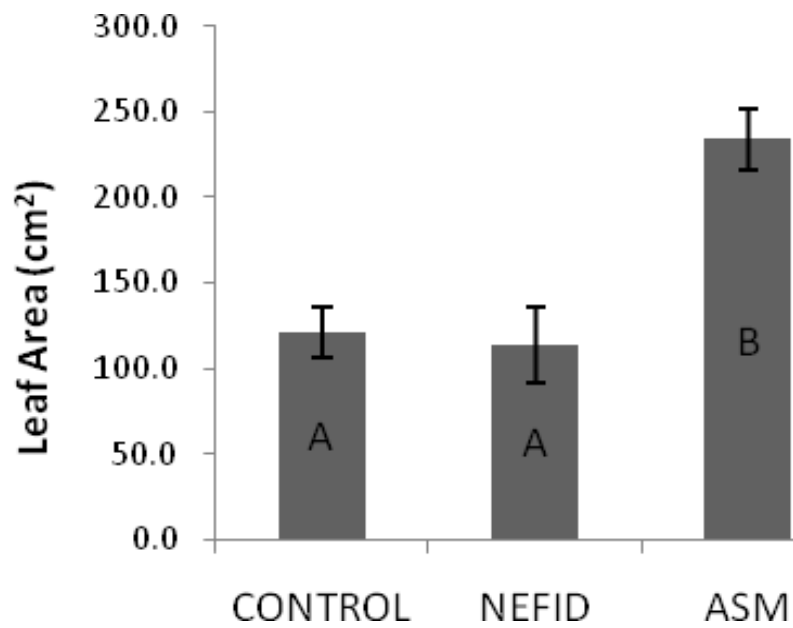


FIGURE 2 Effect of coffee leaves formulation (NEFID) and acibenzolar-S-methyl (ASM) on tomato leaf area. Bars followed by the same letter are not significantly different according to Tukey's multiple range test ($P \leq 0.05$).

TABLE 1 Effect of coffee leaves formulation (NEFID) and acibenzolar-S-methyl (ASM) on tomato seedlings height.

Treatment	Days after spraying				
	3	10	13	16	20
	------(cm)-----				
NEFID	26.70 a	52.83 a	58.62 b	64.42 a	78.33 a
ASM	25.83 a	50.04 a	56.86 b	70.25 b	83.62 b
CONTROL	27.12 a	50.58 a	51.62 a	60.62 a	73.25 a
Mean	26.55	51.15	55.70	65.09	78.40
Ftest_{treatment}	1.32 ^{ns}	0.95 ^{ns}	6.03**	9.93**	17.57**
DMS	2.001	5.296	5.172	5.365	6.490
CV (%)	7.49	10.28	9.22	8.19	8.59

The values were the means of four replicates. Means in the same column followed by the same letter are not significantly different according to Tukey's multiple range test ($P \leq 0.05$); HSD = honestly significant difference; ^{ns}nonsignificant at ($P > 0.05$); CV = coefficient of variation **difference at ($P \leq 0.01$).

3.2 Regulation of PR-1 and beta-1,3-glucanase gene expression by NEFID and ASM.

In order to investigate the similarities in the resistance induction between both tested products, the expression level of key genes (PR-1, β -1,3-glucanase) were investigated over a time course (12, 24, 48, 72, 96, 144 hours). Tomato β -1,3-glucanase gene expression was clearly induced at the earlier stages (12 hours) after spraying the tomato seedlings with NEFID or ASM (3.2 and 1.6 fold changes, respectively) in comparison with the water-sprayed control (Figure 3a). However, treatment with NEFID persisted higher than that of control plantlets at 24, 48, 72 and 144 hours after spraying with 2.02, 2.08,

1.71 and 2.04 fold changes, respectively, while treatment with ASM showed increase on GLU gene expression at 72 and 144 HAS (1.59 and 2.52 fold changes) and no differences compared with control at 24, 48 and 96 HAS.

PR-1 gene expression from NEFID and ASM treated plants initially, at 12 HAS, had demonstrated to be up-regulated with 2.04 and 2.08 fold changes, respectively (Figure 3b). At 24 and 48 HAS there were no differential regulation compared with the water pre-treated plants ($P \leq 0.05$). At 72 and 144 HAS there were a slight difference at PR-1 expression level between NEFID (1.27 and 1.19) or ASM (1.27 and 1.20 fold changes, respectively) for treated and water pre-treated plants.

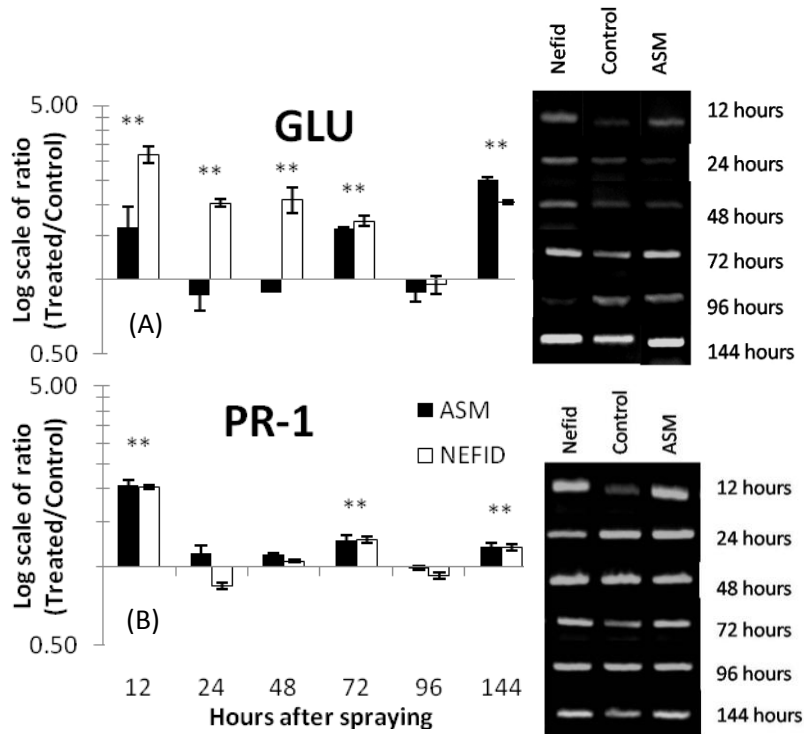


FIGURE 3 ASM and NEFID upregulate GLU and PR-1 levels in tomato. Tomato β -1,3-glucanase gene is upregulated in seedlings sprayed with ASM at 12, 72 and 144 hours after spraying and 12, 24, 48, 72 and 144 hours in plants sprayed with NEFID (a). While PR-1 gene is upregulated at 12, 72 and 144 HAS in both plants sprayed with ASM or NEFID (b). Gene expression ratios are quantified from RT-PCR results. Black bars represent ASM; white bars represent NEFID treatment. $**P \leq 0.01$, treated versus controls (n = 4, mean \pm SD).

3.3 Effects of foliar sprays of NEFID and ASM on the activities of GLU, CHI, PPO and lignin deposition on tomato plants.

Whereas the up-regulation of typical SAR gene (PR-1) was also associated to the expression of chitinase and β -1,3-glucanase genes, post-transcriptional regulation or modulation may affect the overall pathogenesis related protein expression, therefore those enzymes were measured in a 0-240h time-course (Fig. 4). Those studies corroborate gene expression profile, with early protein expression (24-48HAS).

β -1,3-glucanase (GLU) activity at the earliest time point (24 hours) experienced an abrupt increase in values around five and three time higher than the GLU activity of control, for plants pre-treated with ASM and NEFID, respectively (Figure 4a). Treatment of tomato plants with ASM triggered an expression of GLU in all sampled time points which was a similar pattern found for NEFID treatment (induction from 0-120HAS).

Both NEFID and ASM treatments were able to maintain a higher level of chitinase activity at 24HAS. After this early pick, the chitinase activity of tomato plants pre-treated with NEFID or ASM did not differ remarkably from those of plants pre-treated with water (control) for all of after time points, suggesting the tested products did not induce the effect of chitinase for a long time period.

While pathogenesis related enzymes represent an important arsenal for the pathogen control, other pathogen toxic compound synthesis is also triggered by abiotic elicitors such as phenolics and a key enzyme is the polyphenoloxidase (PPO) which was expressed at 24-48HAS for both ASM and NEFID. ASM also induced a later response of this enzyme (240HAS).

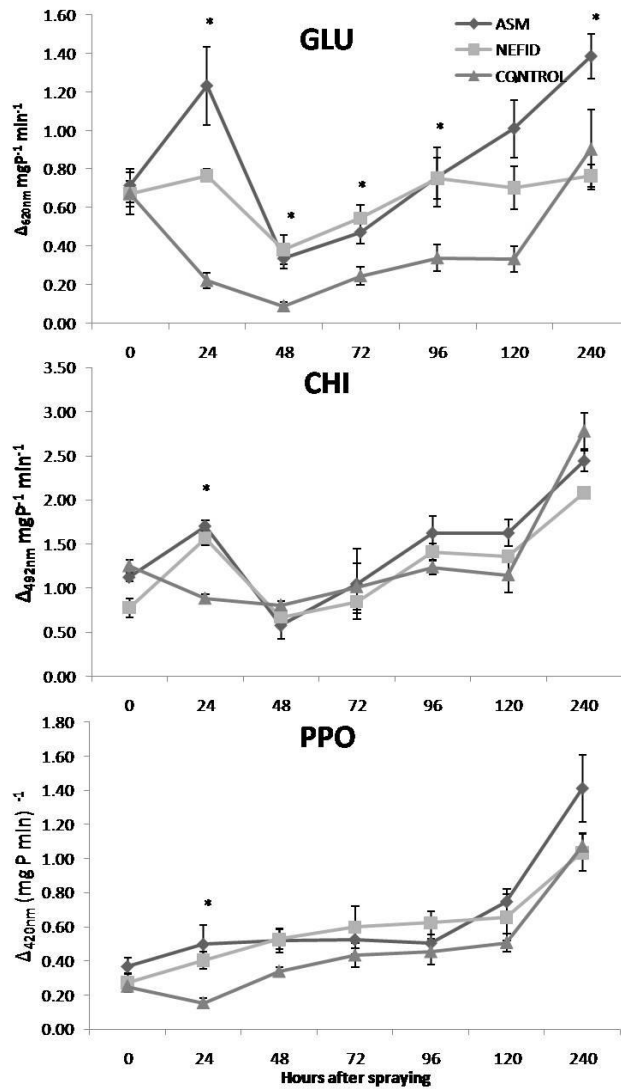


FIGURE 4 Activity of β -1,3-glucanase (GLU) (A), chitinase (CHI) (B) and polyphenol oxidase (PPO) (C) was induced at the earlier stages (24 hours) after spraying the tomato seedlings with NEFID or ASM in comparison with the water-sprayed control. Enzymatic responses were evaluated 0, 24, 48, 72, 96, 120, 240 hours after spraying. Error bars indicate standard deviations.

Although the GLU, CHI and PPO activity of tomato plants pre-treated with ASM, NEFID or water (control), showed significantly ($p \leq 0.05$) different patterns within the tested treatments, the interaction between treatment and pathogen inoculation (*Xanthomonas vesicatoria*) did not show a significant difference, suggesting the bacterium itself in this plant pathogen interaction did not influence the effect of the treatment. In turn, the inoculation with *X. vesicatoria* increases almost two times the GLU activity, at seven days after the inoculation, in tomato plants (data not shown).

Lignin content for ASM and NEFID sprayed plants showed a significant ($p \leq 0.05$) increase in lignin content compared to water-treated controls. The ASM-treated plants showed a significant increase of lignin deposition between 17 and 23 days after spraying in values around two times higher than those measured at the 10-17 DAS interval and higher than the control that remained nearly constant (Figure 5).

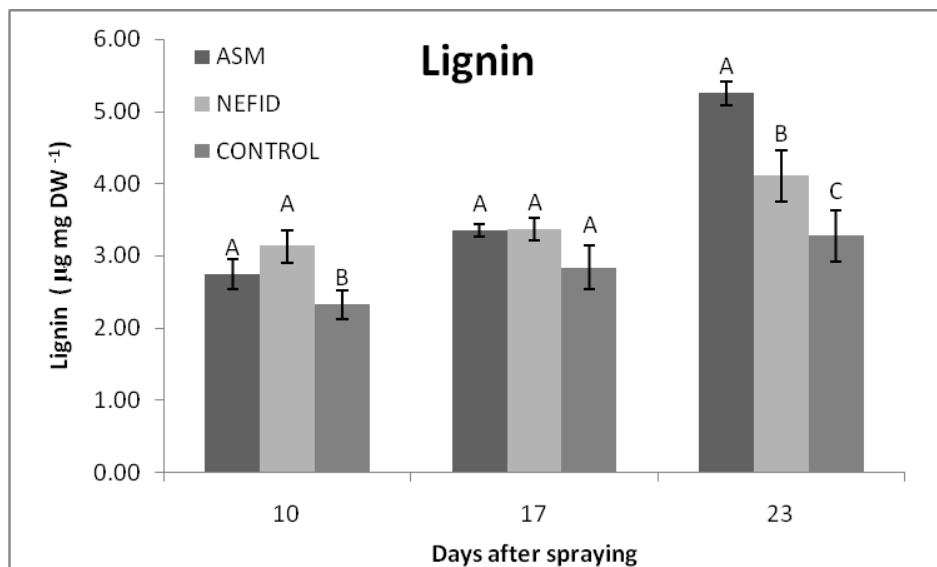


FIGURE 5 ASM and NEFID induced a higher lignin deposition in tomato plants cv. Santa Cruz 'Kada' in comparison with the water-sprayed control. Lignin depositions were evaluated 10, 17 and 23 days after spraying. Error bars indicate standard deviations.

4 DISCUSSION

The worldwide damaging impact of bacterial spot (*Xanthomonas vesicatoria*) on tomato production has resulted in different strategies for plant protection based on fungicides (e.g. mancozeb), biocontrol agents (e.g. *Bacillus sp*) and induced resistance elicitor (e.g. ASM) to be used individually or in combination (Roberts et al., 2008). Induction of systemic acquired resistance (SAR) is generally considered as part of an integrated pest management program because of its broad spectrum and multi-site disease control activity (Da Rocha & Hammerschmidt, 2005). A coffee based leaf formulation (NEFID) exhibits disease reduction properties similar to the commercial product ASM (Figure 1) and comparable to previous findings (Cavalcanti et al., 2006). While ASM and other disease resistance inducers can efficiently control diseases redirecting primary metabolism lowering of biomass accumulation and harvest yields are not always observed (Bostock, 2005). With respect to plant height and leaf surface area over a five point time-course up to 20 days after spraying growth retardation was not observed compared to the untreated control at any time. Furthermore, both ASM and NEFID improved plant height from 13 to 20 das and at 16 das, respectively and ASM improved leaf area.

The mechanisms by which plants detect pathogen presence and initiate a defense response are poorly understood and are the subject of intensive investigation (Anderson et al., 1998). Resistance mechanisms are activated in uninfected tissues (systemic acquired resistance, SAR) which provide the plant more resistant to secondary infection by the same or unrelated pathogens (Ryals et al., 1996). SAR is associated with the increased expression of a wide array of defense genes, among which are those encoding various classes of pathogenesis-related (PR) proteins (Cutt et al., 1992; Stintzi et al., 1993). The functions of some of these proteins during the resistance response

are known, such as the antimicrobial activity of the β -1,3-glucanases (PR-2) and chitinases (PR-3). In contrast, the functions of other PR proteins are less well understood, although they are thought to be integral components of disease resistance. Due to the strict correlation between *PR* gene induction and the development of disease resistance, the *PR* genes are commonly-used molecular markers for the defense response (Anderson et al., 1998).

As expected from previous experiments (Mandal et al., 2008), typical disease resistance responses were present and the SAR marker protein gene PR-1 was up-regulated as early as 12 hours after treatment with ASM and NEFED. Although PR-1's function has not been clearly defined, the up regulation of a SAR marker gene is suggestive of other responses belonging to the same defense pathway being operative. Both chitinase and glucanase are common SAR associated responses and their gene and protein expression levels were investigated. Both were found to be operative for both tested treatments, however in different time-courses. For glucanase, the expression at early time points (up to 96 h) in both treatments (Figure 4a) corroborates with the timeframe necessary from treatment of the elicitors to the inoculation with the pathogen to achieve disease control and was also over-expressed in the induction of resistance against bacterial spot in tomato after treatment with ASM (Cavalcanti et al., 2006). Thus, once the pathogen infects the plant, the defense machinery is ready to hinder the disease onset. The enzyme also has a lysozyme activity acting to hydrolyze bacterial cell walls (Mauch et al., 1988).

In our experiment chitinase was found to be over-expressed in a pattern similar to glucanase (Figure 4a and 4b) even though plants were not challenged with a chitin containing fungal pathogen. The potential of using both ASM and NEFID each one to control fungal diseases has already been reported (Santos et al., 2007) and although NEFID has not been tested against

tomato fungal pathogens, the coffee leaf formulation is expected to be effective in an integrated pest management program against other tomato pathogens.

The use of resistance inducers such as ASM and NEFID activates not only typical SAR responses but also the phenylpropanoid pathway and intermediate compounds such as polyphenols that have a deleterious effect on plant pathogens. It was found to be an early response (24 and 48 h after treatment) but is likely to have a pivotal role in the resistance to bacterial infection. The down-regulation of a gene coding for polyphenol oxidase was correlated with higher *Pseudomonas syringae* infection in tomato (Thipyapong et al., 2005).

One of the products of the phenylpropanoid pathway is the polyphenolic compound lignin which is associated with resistance to bacterial spot in tomato after treatment with ASM (Cavalcanti et al., 2006). This polymer accumulates in the tomato cell wall after treatment with pathogen derived extracts or chitosan delaying the onset of infection and is associated to an early induction of polyphenoloxidase (Mandal & Mitra, 2007).

Both the disease control levels and biochemical responses induced by NEFID and ASM followed a similar pattern with induction of salicylic acid and phenylpropanoid pathways with responses previously reported as being operative against bacterial infection, albeit no adverse effects on the plant development was detected. The observed induction of plant responses associated with growth and defense reported here indicate that the coffee leaf formulation could serve as a biological alternative for tomato growers in the management of bacterial spot with the distinct advantage over ASM in that it can be utilized in organic farming (Santos et al., 2007).

5 REFERENCES

- ACHUO, E. A.; AUDENAERT, K.; MEZIANSH, H. M. The salicylic acid-dependent defense pathway is effective against different pathogens in tomato and tobacco. **Plant Pathology**, Rockville, v. 53, n. 1, p. 65-72, Jan. 2004.
- AL-DAHMANI, J. H.; ABBASI, P. A.; MILLER, S. A.; HOITINK, H. A. J. Suppression of bacterial spot of tomato with foliar sprays of compost extracts under greenhouse and field conditions. **Plant Disease**, Saint Paul, v. 87, n. 8, p. 913-919, Aug. 2003.
- ANDERSON, M. C.; CHEN, Z.; KLESSIG, D. F. Possible involvement of lipid peroxidation in salicylic acid-mediated induction of *pr-1* gene expression. **Phytochemistry**, Oxford, v. 47, n. 4, p. 555-566, Feb. 1998.
- ANTEROLA, A. M.; LEWIS, N. G. Trends in lignin modification: a comprehensive analysis of the effects of genetic manipulations/mutations on lignification and vascular integrity. **Phytochemistry**, Oxford, v. 61, n. 3, p. 221-294, Oct. 2002.
- BOKSHI, A. I.; MORRIS, S. C.; DEVERALL, B. J. Effects of benzothiadiazole and acetylsalicylic acid on beta-1,3-glucanase activity and disease resistance in potato. **Plant Pathology**, Oxon, v. 52, n. 1, p. 22-27, Feb. 2003.
- BOSTOCK, R. M. Signal cross-talk and induced resistance: straddling the line between cost and benefit. **Annual Review of Phytopathology**, Palo Alto, v. 43, n. 1, p. 545-580, Jan. 2005.
- BRADFORD, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, San Diego, v. 72, n. 1/2, p. 248-254, Jan. 1976.
- CARMO, M. G. F.; CORREA, F. M.; CORDEIRO, E. S.; CARVALHO, A. O.; ROSSETTO, C. A. V. Eradication treatments of *Xanthomonas vesicatoria* and its effect on the quality of tomato seeds. **Horticultura Brasileira**, Brasília, DF, v. 22, n. 3, p. 579-584, Mar. 2004.

CARMO, M. G. F.; KIMURA, O.; MAFFIA, L. A.; CARVALHO, A. O. Determinação do nível de tolerância de *Xanthomonas campestris* pv. *vesicatoria* em sementes de pimentão. **Fitopatologia Brasileira**, Brasília, DF, v. 20, n. 2, p. 336-341, mar./abr. 1996.

CAVALCANTI, F. R.; RESENDE, M. L. V.; CARVALHO, C. P.; SILVEIRA, J. A.; OLIVEIRA, J. T. An aqueous suspension of *Crinipellis pernicioso* mycelium activates tomato defence responses against *Xanthomonas vesicatoria*. **Crop Protection**, Oxford, v. 26, n. 5, p. 729-738, May 2007.

CAVALCANTI, F. R.; RESENDE, M. L. V.; ZACARONI, A. B.; RIBEIRO JÚNIOR, P. M.; COSTA, J. C. B.; SOUZA, R. M. Acibenzolar-S-methyl and Ecolife[®] in the induction of defense responses in tomato against bacterial spot (*Xanthomonas vesicatoria*). **Fitopatologia Brasileira**, Lavras, v. 31, n. 4, p. 372-80, ago./set. 2006.

GAUILLARD, F.; RICHARD-FORGET, F.; NICOLAS, J. New spectrophotometric assay for polyphenol oxidase activity. **Analytical Biochemistry**, San Diego, v. 215, n. 1, p. 59-65, Nov. 1993.

HAMMOND-KOSACK, K. E.; PARKER, J. E. Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. **Current Opinion in Biotechnology**, London, v. 14, n. 2, p. 177-193, Apr. 2003.

HE, C. Y.; HSIANG, T.; WOLYN, D. J. Induction of systemic disease resistance and pathogen defence responses in *Asparagus officinalis* with nonpathogenic strains of *Fusarium oxysporum*. **Plant Pathology**, Oxford, v. 51, n. 2, p. 225-230, Apr. 2002.

LAWTON, K. A.; FRIEDRICH, L.; HUNT, M.; WEYMANN, K.; DELANEY, T.; KESSMANN, H.; STAUB, T.; RYALS, J. Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. **Plant Journal**, Oxford, v. 10, n. 1, p. 71-82, July 1996.

LOON, L. C. van; STRIEN, E. A. van. The families of pathogenesis related proteins, their activities, and comparative analysis of PR-1 type proteins. **Physiological and Molecular Plant Pathology**, London, v. 55, n. 2, p. 85-97, Aug. 1999.

MANDAL, A.; MITRA, S. Reinforcement of cell wall in roots of *Lycopersicon esculentum* through induction of phenolic compounds and lignin by elicitors. **Physiological and Molecular Plant Pathology**, London, v. 71, n. 4/6, p. 201-209, Oct./Dec. 2007.

MANDAL, B.; MANDAL, S.; CSINOS, A. S.; MARTINEZ, N.; CULBREATH, A. K.; PAPPU, H. R. Biological and molecular analysis of the acibenzolar-S-methyl induced systemic acquired resistance in flue cured tobacco against tomato spotted wilt virus. **Phytopathology**, Saint Paul, v. 98, n. 2, p. 196-204, Feb. 2008.

MAUCH, F.; MAUCH-MANI, B.; BOLLER, T. Antifungal hydrolases in pea tissue: II., inhibition of fungal growth by combinations of chitinase and beta-1,3-glucanase. **Plant Physiology**, Rockville, v. 88, n. 3, p. 936-942, Nov. 1988.

MELLO, S. C. M.; TAKATSU, A.; LOPES, C. A. Escala diagramática para avaliação da mancha-bacteriana do tomateiro. **Fitopatologia Brasileira**, Lavras, v. 22, n. 3, p. 447-448, maio/jun. 1997.

MONTIES, B. Lignins. In: DEY, P. M.; HARBORNE, J. B. (Ed.). **Methods in plant biochemistry**. New York: Academic, 1989. v. 1, p. 113-158.

NÜRNBERGER, T.; BRUNNER, F. Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecules. **Current Opinion in Plant Biology**, London, v. 5, n. 4, p. 318-324, Aug. 2002.

ROBERTSA, P. D.; MOMOLB, M. T.; RITCHIEB, L.; OLSONB, S. M.; JONESC, J. B.; BALOGH, B. Evaluation of spray programs containing famoxadone plus cymoxanil, acibenzolar-S-methyl, and *Bacillus subtilis* compared to copper sprays for management of bacterial spot on tomato. **Crop Protection**, Oxford, v. 27, n. 12, p. 1519-1526, Dec. 2008.

ROCHA, A. B. da; HAMMERSCHMIDT, R. Historical perspectives on the use of disease resistance inducers in horticultural crops. **HortTechnology**, Alexandria, v. 15, n. 3, p. 518-529, Sept. 2005.

RYALS, J. A.; NEUENSCHWANDER, U. H.; WILLITS, M. G.; MOLINA, A.; STEINER, H. Y.; HUNT, M. D. Systemic acquired resistance. **The Plant Cell**, Rockville, v. 8, n. 10, p. 1809-1819, Oct. 1996.

SANTOS, F. S.; SOUZA, P. E.; RESENDE, M. L. V.; POZZA, E. A.; MIRANDA, J. C.; RIBEIRO JÚNIOR, P. M.; MANERBA, F. C. Efeito de extratos vegetais no progresso de doenças foliares do cafeeiro orgânico. **Fitopatologia Brasileira**, Lavras, v. 32, n. 1, p. 59-63, fev. 2007.

SOYLU, S.; BAYSAL, O.; SOYLU, E. M. Induction of disease resistance by the plant activator, acibenzolar-S-methyl (ASM), against bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) in tomato seedlings. **Plant Science**, Clare, v. 165, n. 5, p. 1069-1075, Nov. 2003.

STINTZI, A.; HEITZ, T.; PRASAD, V.; WIEDEMANN-MERDINOGLU, S.; KAUFFMANN, S.; GEOFFROY, P.; LEGRAND, M.; FRITIG, B. Plant 'pathogenesis-related' proteins and their role in defense against pathogens. **Biochimie**, Paris, v. 75, n. 8, p. 687-706, Jan. 1993.

THANGAVELU, R.; PALANISWAMI, A.; DORAISWAMY, S.; VELAZHAHAN, R. The effect of *Pseudomonas fluorescens* and *Fusarium oxysporum* f. sp. *cubense* on induction of defense enzymes and phenolics in banana. **Biology Plantarum**, Dordrecht, v. 46, n. 1, p. 107-112, Jan. 2003.

THIPYAPONG, P.; HUNT, M.; STEFFEN, J. C. Antisense down-regulation of polyphenol oxidase results in enhanced disease susceptibility. **Planta**, New York, v. 220, n. 1, p. 105-117, Nov. 2004.

UNIVERSIDADE FEDERAL DE LAVRAS. RESENDE, M. L. V.; CAVALCANTI, F. R.; SANTOS, F. S.; RIBEIRO JUNIOR, P. M.; AMARAL, D. R. **Formulação para indução de resistência em plantas, a base de extrato vegetal obtido de folhas do cafeeiro**. BR n. INPI 0000220604167501. 2 ago. 2006.

CHAPTER 3

Defense gene expression induced by the biocontrol coffee-leaf formulation in tomato

ABSTRACT

Plant formulations have the potential to activate defense related genes and the mechanism of action can be monitored in plants by using microarray technology. A coffee leaf formulation has shown its potential in the control of both bacterial and fungal pathogens in different hosts. The transcriptional response of coffee leaf elicitor (NEFID) in plants was evaluated by using a tomato gene chip. A total of 268 genes were found to be differentially regulated with a majority up-regulated and encoding signal transduction, defense responses, and transcription factors. Since salicylic- and jasmonic-acid signaling components were not differentially transcribed with elicitor treatment while mitogen activated proteins (MAP3K and MAPKK) as well as calcium dependent (calmodulin and phosphatidylinositol) signaling components were up regulated, a SA/JA-independent transduction sequence for PR accumulation is predicted. Chitinases, glucanases and peroxidases (PR-4), with reported activity against pathogens, were transcriptionally up-regulated and corresponding enzyme activities were over-expressed as early as 24 h post treatment and remained elevated for up to five days subsequently. These results demonstrate the ability of the coffee leaf formulation (NEFID) to differentially regulate gene expression in tomato.

Keywords: *Solanum lycopersicum*, systemic acquired resistance, natural formulation, microarray approach

RESUMO

Formulações de plantas tem o potencial de ativar genes relacionados a defesa e o seu mecanismo de ação pode ser monitorado nas plantas utilizando-se a tecnologia dos microarranjos. Uma formulação a base de folhas de café tem demonstrado potencial de controle de patógenos bacterianos e fúngicos em diferentes hospedeiros. A resposta transcricional em plantas de tomateiro mediada por uma formulação baseada em folhas de café (NEFID) foi avaliada utilizando-se um chip de microarranjos de tomate. Um total de 268 genes foram diferencialmente regulados, em sua maioria super-expressos, codificando principalmente para a transdução do sinal, resposta de defesa e fatores de transcrição. Uma vez que componentes sinalizadores da rota do ácido salicílico e jasmônico não foram diferencialmente transcritos com o tratamento pelo eliciador (NEFID) enquanto proteínas ativadas por mitógenos (MAP3K e MAPKK) assim como componente sinalizadores dependentes de cálcio (calmodulina e fosfatidilinositol) foram super-expressos, uma sequência de transdução, independente de SA/JA, para a acumulação de PR proteínas é predita. Quitinases, glucanases e peroxidases (PR-4), os quais tem atividade antipatogênica relatada, foram transcricionalmente super-expressas e atividades enzimáticas correspondentes as estas enzimas foram também super-expressas 24 horas após a pulverização do eliciador e manteve-se elevada por até cinco dias subsequentes. Estes resultados demonstram a habilidade da formulação à base de folhas de café em regular a expressão gênica em tomateiro.

Palavras-chave: *Solanum lycopersicum*, resistência sistêmica, formulação natural, técnica de microarranjos

1 INTRODUCTION

Tomato (*Solanum lycopersicum* L., previously *Lycopersicon esculentum* Miller) is among the economically most important crops worldwide (Souza & Resende, 2003). Furthermore the plant species is diploid, has a short generation time, a feasible transformation technology, rich available genomic information and therefore represents a model plant for molecular biology studies (Barone et al., 2008). The crop is affected by over 200 pathogens (Jones et al., 1991) and researchers have directed efforts towards tomato disease control, especially bacterial spot, caused by *Xanthomonas euvericatoria* (Wang et al., 2004).

The disease control relies on the use of agrochemicals and plant resistance. Although the main agrochemicals streptomycin sulfate and copper-based products have proven to be efficient, due to its overuse, resistant populations have arisen (Gore & Garro, 1999). From a recent breeding program, a tomato cultivar has already achieved resistance to the different pathogen races (Souza et al., 2008) but the breeding line is not yet available to growers and it is not impossible that a new race not considered in the breeding would lead to failure in the disease control from the exclusive use of bacterial spot resistant plants.

Alternatively, disease control may be achieved by the elicitation of plant resistance genes prior to pathogen attack. Several products have shown elicitation activity such as the salicylic acid analogue acibenzolar-S-methyl (ASM). When tomato plants were sprayed with ASM in the field where bacterial spot occurred, an increase of up to 134% in fruit yield was observed and the results were comparable to the standard bactericide (Louws et al., 2001).

Other elicitors may be based on inactivated pathogen (Cavalcanti et al., 2007) or even a combination of both inactivated pathogen and plant oligomers, and a product based on rust infected coffee leaf formulation, so-called NEFID, has recently been patented (I.N.P.I., Protocol number 0000220604167501, “Formulation for resistance induction”... Aug/2006) that contains both elicitors. NEFID was effective in the control of coffee rust and phoma spot, bacterial blight in cotton as well as bacterial spot in tomato. The underlying mechanism was the accumulation of pathogenesis-related proteins, phytoalexin production and lignin content in the leaves following plant sprays (Barguil et al., 2005, Ishida et al., 2007; Santos et al., 2007).

An important strategy to probe not only the defense-related events but all changes in the plant metabolism due to an elicitor treatment is the use of microarrays (Moore et al., 2005). When challenged with a yeast elicitor, tomato was found to induce SAR-dependent PRs (chitinase and glucanase), an up-regulation of cytochrome P450 and a down-regulation of cell wall loosening and expansion (expansin, xyloglucan endoglycosyl transferase, polygalacturonase) (Jiang et al., 2009). SAR-elicited plants were yet involved in acclimatory responses to stress, such as recovery of the cell redox balance (glutathione transferase, UDP glycosyl transferase and glutaredoxins), intracellular stress signaling, improvement of pathogen recognition, and promotion of metabolic changes (Blanco et al., 2008). On the other hand, plants treated with fusicoccin displayed an elicitation of plant defense genes as well as the expression of a jasmonic acid biosynthesis gene which was not related to the defense signaling but associated to the inhibition of the biosynthesis of photosynthetic pigments and photosynthetic activity (Frick & Schaller, 2002).

The plant formulation NEFID when sprayed on tomato plants protected them against bacterial spot and we have characterized the overall transcriptomics change after treatment using the *Solanum lycopersicon*

oligomer microarray chip TOM2. In this study we found a changed regulation of 268 genes and report that bacterial treatment induces a SA or JA independent activation of PR genes (chitinase, glucanase and peroxidase) as well as an over-expression of the corresponding proteins as early as 24h after treatment. We also addressed the other genes with changed regulation such as cell wall modification, transcription factor and stress alleviation genes and the the possible impacts on phenotype.

2 MATERIAL AND METHODS

2.1 Plant material and NEFID formulation

Tomato seeds (*Solanum lycopersicum* var. Santa Cruz Kada), purchased from Isla Sementes Ltda (Porto Alegre — Rio Grande do Sul, Brazil) were surface sterilized in 1% (v/v) ethanol for 3 min, followed by a 1.0 g L⁻¹ sodium hypochlorite solution for 1 min and then rinsed thorough with distilled water. Seeds were sown in 1.5 L pots containing 400 g of the potting mix Sunshine® All-Purpose Planting Mix (Sun Gro Horticulture, Vancouver, CA), fertilized with 5 g of Osmocote fertilizer (Scotts-Sierra Horticulture, Marysville, OH, USA), irrigated to field capacity daily. Plants were grown under a controlled temperature (25° C ± 4), relative humidity 40 ± 10% and light (200 μmol m⁻² s⁻¹) by using a combination of metal halide and high sodium pressure lamps set for 14 h/day light period. For enzyme assays and disease severity measurements, plants were greenhouse grown in Plantmax® soil (Eucatex, Paulinia, SP, Brazil) with a mean temperature of 28 ± 3° C day/23 ± 3° C night, relative humidity of 40 ± 3%/85 ± 3%, and a 12 h photoperiod, at 450–500 mmol m⁻² s⁻¹ maximum photon flux densities, measured at plant level (IRGA, model LCA-4, Hoddesdon, UK).

The coffee formulation (NEFID) contains field-collected coffee leaves (*Coffea arabica*), from soil surface (due to disease, harvest fruit and/or other stresses) and selected for powder production. A hundred grams of this powder is mixed with 1000 mL distilled water, boiled in reflux and filtered through a sieve of 400 meshes. The filtrate of leaves is sampled and stored at -20°C. The formulation based on NEFID is patented by Resende et al. (2006) **(I.N.P.I., Protocolo número 0000220604167501, FORMULAÇÃO PARA INDUÇÃO DE RESISTÊNCIA... 02 de Agosto de 2006)**. Twenty-five days

after planting, (plant height ca. 20 ± 4 cm), tomato leaves were sprayed with either NEFID or H₂O (control).

2.2 Plant sampling and RNA extraction

Plant tissue was harvested and frozen in liquid nitrogen and stored at -80°C for RNA extraction.

Samples were ground in mortar and pestle under liquid nitrogen and RNA extracted following the protocol of RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) including the RNase-free DNase treatment step from the same manufacturer. The clean RNA was quantified and stored at -80°C until use.

2.3 Microarray analysis

Microarray hybridizations consisted of four biological replicates, one of which consisted of a dye swapping. The previously obtained target RNA was transcribed to aRNA in a three step transcription using Amino-Allyl aRNA Amplification Kit (Ambion, Austin, TX, USA) and labeled with NHS dyes Cy3 or Cy5 (Amersham Biosciences, Little Chalfont Buckinghamshire, UK) according to the manufacturer's protocol. Samples were assayed on the tomato TOM2 oligo-arrays printed at University of Arizona. Each chip containing 12,160 70-mer oligonucleotide elements (<http://ted.bti.cornell.edu/cgi-bin/TFGD/array>).

Slide pre-hybridization was performed according to the manufacturer, whereas hybridization and post-hybridization followed the *Arabidopsis thaliana* protocol (Zhang et al., 2007). The arrays were scanned using a GenePix 4100 array scanner (Axon Instruments, Sunnyvale, CA, USA). Spot statistical analysis was performed according to the manufacturer's guidelines (Gene-Spring 7.0; Silicon Genetics, Redwood, CA, USA). A 40% change, either up- or down-regulation, in the expression level compared with the control

was selected as the threshold for a gene to be classified as altered in response to NEFID treatment. Only genes that passed the Flag Filtering, identified as present (Gene-Spring 7.0), and passed the T-test P-values 0.10 was considered differentially regulated with the NEFID treatment.

2.4 Validation of microarray result

For the microarray result validation, first strand cDNA was synthesized from 5µg of total RNA following Zhang et al. (2007) and PCR performed using the (5'-3') primers designed based on genes with significantly changed expression (Table 1). Seven genes were chosen (Table 1) and the primers designed based on the UNIGENE used to generate each microarray probe (Tomato functional genomics database, available at <http://ted.bti.cornell.edu/>).

TABLE 1 Primers used for RT-PCR

Gene	Name	Forward	Reverse
Endo-1,3-beta-glucanase	SGN-U212943	ACAGCTCATACATGGC CTTCT	ATTGGGCTTCTTGGTTG TGGTTGG
Pectinesterase	SGN-U214672	CGCATGGGCTGATTGC ATTGAACT	CGACGACCGACGATGC AACAAATT
Chitinase	SGN-U224778	ATGGCGGAAACTGTCC TAGTGGAA	ACATGGTCTACCATCAG CTTGCCA
Calmodulin	SGN-U212854	TGCTGGTAGTTGTGGG AGTTGAGA	AGCTCCTTAGTCGTGAT GCAACCT
Peroxidase	SGN-U213351	ACGGAGCAAGCGACA ATTGACAAC	CGATTGATTCACCGCAA AGCTCGT
Proteinase inhibitor	SGN-U213363	CGGAGAATCTGAATGG GTAAGCGA	ACAAGCCGTGGTAAAG GTCCACAA
Glutathione transferase	S- SGN-U216884	TGTCCCAACCTTCTCGT GCAGTTA	TGAGTGATGCCAGTCCA ACACAGA
Actin	SGN-U226051	TTGACTGAGGCACCAC TTAACCCT	GCTTTCAGGTGGTGCAA CGACTTT

Agarose gel electrophoresis images were taken by Kodak Gel Logic 100 Imaging System (Fisher Scientific, Houston, TX, USA) and the band intensity quantified by Image J 1.33u (<http://rsb.info.nih.gov/ij/>, National Institute of Health, USA).

2.5 Enzyme extraction, PR-protein, defense enzyme assay

Tomato fresh leaf (1.0 g), treated with the natural formulation (NEFID) and control (water sprayed), collected at 0, 24, 48, 72, 96 and 120 hours after spraying (HAS), was homogenized for 5 min in a mortar with a pestle in 3 mL of ice-cold 50mM sodium acetate buffer pH 5.2, containing 0.1mM EDTA. After filtration, the homogenate was centrifuged at 13,000g for 15 min and the supernatant (crude extract) used as the source of enzymes. All the steps were carried out at 0–4° C (Cavalcanti et al., 2007). Protein content of the crude extracts was determined using the Bradford (1976) protein assay, with bovine serum albumin (BSA) as a standard.

The activity of guaiacol peroxidase POX (EC 1.11.1.7) was determined by adding 25 mL of the crude-extract preparation to 2mL of a solution containing 50mM sodium acetate buffer, pH 5.2, 20mM guaiacol and 20mM hydrogen peroxide. After incubation at 30° C for 10 min, the absorbance was read at 480nm (Urbanek et al., 1991). One POX unit of activity (UA) was expressed as the variation of one unit of absorbance at 480nm per milligram of soluble protein per minute (UA mgP⁻¹ min⁻¹).

Chitinase activity CHI (PR-3; EC 3.2.1.14) was determined by adding 70 µL of suitably diluted crude extract with 130 µL of 50 mM sodium acetate pH 5.2 and 60 µL of CM-Chitin-RBV (2 mg mL⁻¹), a polymeric carboxymethyl-substituted chitin, labelled covalently with Remazol Brilliant Violet 5R (CM-Chitin-RBV, Loewe, Biochemica, Germany) used as substrate, in microplates of 96 wells with a capacity of 350 µL. After incubation at 35° C

for 80 min, samples were acidified with 50 μL of 0.5 N HCl, cooled in ice bath for 10 min and centrifuged (1,450 g for 10 min). Absorbance of the supernatant at 492nm was recorded and the results were expressed as UA. One unit of CHI activity was defined as the variation of one absorbance unit at 492 nm per milligram of soluble protein per minute ($\text{UA mgP}^{-1} \text{min}^{-1}$). Assays were carried out in triplicate.

The activity of beta-1,3-glucanase GLU (PR-2; EC 3.2.1.39) was measured using similar method, with the exchange of the substrate for CM-Curdlan-RBB (4mg mL^{-1}) and the adjustment of the rate of enzyme extract to $100\mu\text{L}$ (minus the volume of acetate buffer in order to adjust the final volume to $310\mu\text{L}$ per cavity). To promote the hydrolytic action of beta-1 3-glucanase was adopted incubation period of 35°C for 100 min. Absorbance of the supernatant at 620 nm was recorded and the results were expressed as UA. One unit of CHI activity was defined as the variation of one absorbance unit at 620 nm per milligram of soluble protein per minute ($\text{UA mgP}^{-1} \text{min}^{-1}$). Assays were carried out in triplicate.

2.6 Experimental design and statistical methods

For the biochemical determinations, experiments were arranged in randomized block designs, with three blocks, one experimental unit (plot) consisted of four 3 L pot containing three plants each. For analysis by microarray and reverse transcription–polymerase chain reaction (RT–PCR) experiments, plants were arranged in randomized blocks designs, with three blocks, and one experimental unit (plot) consisted of four 1.5 L pot containing two plants each. Variance analysis was run using SAS (Statistical Analysis Systems Inc., Cary, NC, USA) statistical software. Means were separated using Tukey’s test at P value less than 0.05 using Sisvar, a statistical tool purchased from the Federal University of Lavras, Minas Gerais, Brazil.

3 RESULTS

3.1 Regulation of gene expression by NEFID

In order to provide insight into underlying mechanisms responsible for the induction of resistance by a natural formulation based in *Coffea arabica* leaves, genome-wide analysis of gene expression was performed using oligonucleotide microarray slides. Three independent microarray analyses were executed. Labeled mRNA from tomato leaves tissue harvested at 12 hours after spraying with the natural formulation of coffee leaves (NEFID) or water was hybridized with microarray slides, with 12,000 spots, designed for over 11,000 tomato unigenes. Changes in RNA levels in response to NEFID treatment (i.e. induced or repressed) were assessed using oligo microarray slides (TOM2).

A total of 268 genes were differentially expressed with NEFID treatment compared to the water control, most of which (80%) were up-regulated. The number of genes with increased signals was 215 and that with decreased signals was 53. Microarray responses were validated by RT-PCR analysis of selected genes. All seven genes tested showed a similar fold change (Figure 1).

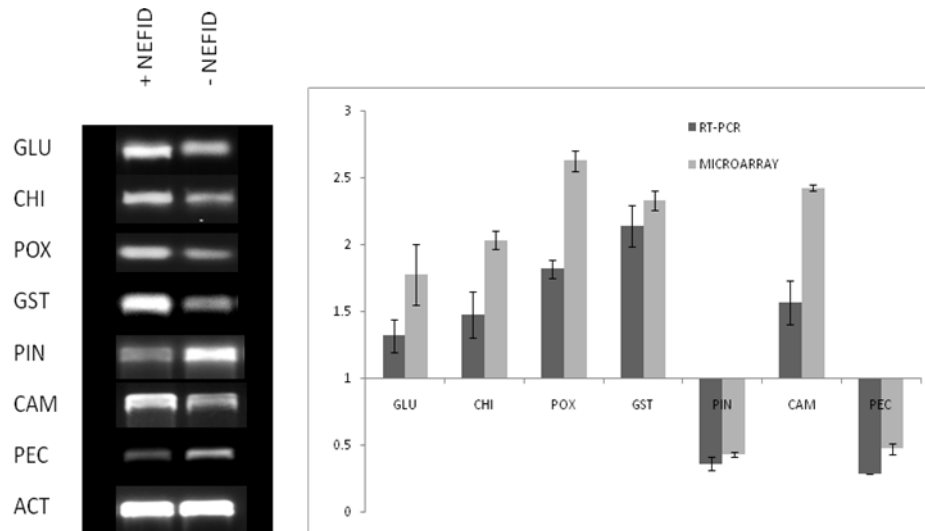


FIGURE 1 Expression level of genes of significantly changed regulation ($P < 0.10$ and ratio > 1.4 or < 0.6) from the microarray data compared to the RT-PCR: endo 1,3 beta-glucanase SGN-U212943 (GLU), Chitinase SGN-U224778 (CHI), Peroxidase SGN-U213351 (POX), Glutathione S-transferase SGN-U216884 (GST), Proteinase inhibitor SGN-U213363 (PIN), Calmodulin SGN-U212854 (CAM), Pectinesterase SGN-U214672 (PEC) e Actin (ACT).

3.2 Putative identification and functional category assignment

The putatively known genes for which significant expression changes were observed and tentatively arranged in categories according to its reported function. The putative function of the NEFID-regulated genes fits into the following categories, “signal transduction” (9% of up-regulated genes, 2% of down-regulated genes), “transcription factor” (8% of up-regulated genes, 11% of down-regulated genes), “oxidative burst/hypersensitive response” (7% of up-regulated genes), “defense response” (6% of up-regulated genes, 4% of down-regulated genes), “energy pathways” (6% of up-regulated genes, 4% of down-regulated genes), “protein biosynthesis (5% of up-regulated genes, 2% of

down-regulated genes), “protein degradation” (4% of up-regulated genes), “cell wall” (4% of up-regulated genes, 4% of down-regulated genes), “cell structure” (2% of up-regulated genes, 5% of down-regulated genes), “metabolism” (2% of up-regulated genes, 2% of down-regulated genes), “hormone response” (2% of up-regulated genes, 4% of down-regulated genes), “transport” (2% of up-regulated genes, 2% of down-regulated genes), “lipid metabolism” (2% of up-regulated genes, 4% of down-regulated genes), “photosynthesis” (1% of up-regulated genes, 19% of down-regulated genes), “nucleic acid metabolism” (2% of up-regulated genes), “G protein” (2% of up-regulated genes), “stress response” (2% of up-regulated genes), “detoxification” (1% of up-regulated genes), “proteinase inhibitor” (13% of down-regulated genes), “others” (9% of up-regulated genes, 11% of down-regulated genes), “unknown” (23% of up-regulated genes, 13% of down-regulated genes) as shown in Figure 2.

The five categories with the larger number of representatives (except for unclassified protein) were the up-regulated signal transduction (with 7.5% of the regulated genes total), transcription factors (6.3%), oxidative burst/hypersensitive response (5.2%), defense response (4.8%) and energy pathways (4.5%) their counterparts found to be down-regulated were much less abundant with 0.4, 2.2, 0.0, 0.7, 0.7% of the regulated genes total, respectively. The category with the larger number of representatives in the down-regulated genes was the photosynthesis related genes with 10 genes suppressed.

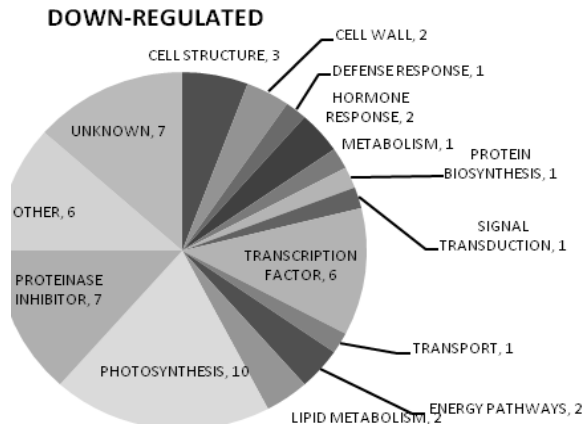
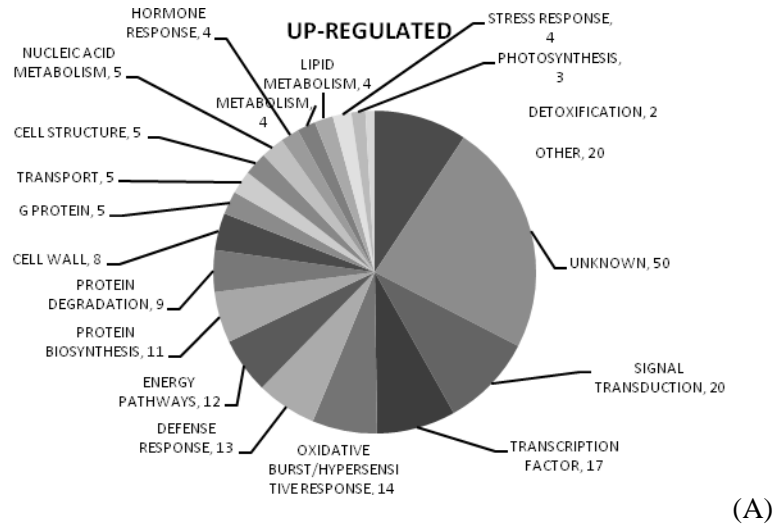


FIGURE 2 Pie charts showing the number of (A) (up-regulated genes) and (B) (down-regulated genes) in each of the functional categories.

TABLE 2 Classification of gene differentially expressed in *Solanum lycopersicum* 12 hours after NEFID treatment.

Gene classes	Gene number	Response	Ratio (treated/control)
Up-Regulated = 215 genes – 80% of regulated genes total			
SIGNAL TRANSDUCTION	20	calcium-dependent protein kinase, calmodulin-binding protein, transducin / WD-40 repeat protein, calmodulin, protein kinase, phototropic response protein, phosphatidylinositol-4-phosphate 5-kinase, MAP3K-like protein kinase, mitogen-activated protein kinase kinase (MAPKK), leucine rich repeat protein family contains protein kinase domain, receptor-related serine/threonine kinase, protein phosphatase 2C (PP2C), tyrosine phosphatase, calcineurin-like phosphoesterase, F-box protein family similar to SKP1 interacting partner 2 (SKIP2), transmembrane protein	1.48-2.42
TRANSCRIPTION FACTOR	17	HD-Zip transcription factor Athb-13, bZip DNA binding protein, homeobox-leucine zipper protein HAT5, DHHC-type zinc finger domain, transcription factor L2, MADS-box family protein, homeobox-leucine zipper protein ATHB-13, MADS-box protein 9, MYB family transcription factor, transcription factor SF3, transcriptional factor B3 family protein / auxin-responsive factor, GT-1-related transcription factor, transcriptional adaptor like protein, homeodomain protein contains 'Homeobox' domain signature,	1.40-2.56
OXIDATIVE BURST/HYPERSENSITIVE RESPONSE	14	peroxidase, glutathione peroxidase, copper/zinc superoxide dismutase (CSD2), copper/zinc superoxidase dismutase (CSD1), glyoxalase II, cytochrome P450, glutathione transferase,	1.41-2.62
DEFENSE RESPONSE	13	Pathogenesis-related protein 1 precursor (PR-1), endo-1,3-beta-glucanase-like protein, basic endochitinase, hevein-related protein precursor (PR-4), pathogenesis-related protein, glycosyl hydrolase family 19 (basic endochitinase), disease resistance protein (NBS-LRR class), leucine rich repeat protein, (PDF2.3) plant defensin protein, disease resistance protein, VIP2 protein, TSW12, Ethylene-responsive proteinase inhibitor I precursor,	1.45-2.88
ENERGY PATHWAYS	12	pyruvate, orthophosphate dikinase, lycopene beta cyclase, malate oxidoreductase (NADP-dependent malic enzyme), gamma-VPE (vacuolar processing enzyme), GCN5-related N-acetyltransferase (GNAT), short-chain dehydrogenase/reductase, mitochondrial aldehyde dehydrogenase (ALDH3), GDP-mannose pyrophosphorylase, L-allo-threonine aldolase, epsilon subunit of mitochondrial F1-ATPase, cytochrome b561-related, cytochrome b5 domain-containing protein	1.47-2.34

... continued...

TABLE 2 Cont.

PROTEIN BIOSYNTHESIS	11	ubiquitin family, deoxyhypusine synthase, cytosolic cyclophilin (ROC3), cyclophilin ROC7, 40S ribosomal protein S14 (RPS14B), 60S ribosomal protein L10A (RPL10aB), 60S acidic ribosomal protein P0 (RPP0B), symbiosis-related like protein, RHO GDP-dissociation inhibitor 1 -related, eukaryotic rpb5 RNA polymerase subunit, eukaryotic translation initiation factor 4A-1 (eIF4A-1)	1.40-1.81
PROTEIN DEGRADATION	9	serine carboxypeptidase, serine carboxypeptidase III, Ethylene-responsive proteinase inhibitor I precursor, proteasome regulatory particle triple-A ATPase subunit4, ubiquitin-conjugating enzyme 2 (UBC2) E2, ubiquitin-associated (UBA)/PB1, ubiquitin-conjugating enzyme 10 (UBC10) E2,	1.54-2.36
CELL WALL	8	endo-1,4-beta-glucanase, cellulase, xyloglucan endotransglycosylase, glycosyltransferase family 8, xyloglucan endo-1,4-beta-D-glucanase (EC 3.2.1.-) precursor, alpha-expansin 6 precursor, Alpha 1,4-glycosyltransferase, O-diphenol-O-methyl transferase,	1.41-2.17
G PROTEIN	5	GTPase activating protein, ARF GTPase-activating domain, GTP-binding protein, Ras-related GTP-binding protein (ARA-4), putative ATP(GTP)-binding protein	1.53-1.87
TRANSPORT	5	ATPase plasma membrane-type (proton pump), H ⁺ -transporting ATP synthase-related protein, SNF7 protein, putative UDP-galactose transporter, transporter - related low similarity to hexose transporter	1.46-1.87
CELL STRUCTURE	5	histone H2A, profilin 5, actin polymerisation complex protein, actin-related protein 8B (ARP8) protein	1.51-1.68
NUCLEIC ACID METABOLISM	5	Macrophage migration inhibitory factor (MIF), RNA-binding protein, endonuclease/exonuclease/phosphatase family, ADP-ribosylation factor, RNA recognition motif (RRM)	1.43-2.04
HORMONE RESPONSE	4	auxin-responsive protein, ethylene-response protein ETR1, arginine decarboxylase, 2-oxoglutarate-dependent dioxygenase	1.47-2.59
METABOLISM	4	glutamate decarboxylase (EC 4.1.1.15) 2, starch synthase, phosphomannomutase -related, phytoene synthetase	1.65-2.00
LIPID METABOLISM	4	lipase (class 3) family, ceramidase family protein, myo-inositol-1-phosphate synthase -related protein, thioesterase family	1.55-1.75
STRESS RESPONSE	4	heat shock protein, Pi starvation-induced protein, metallothionein 2b, DnaJ protein	1.43-1.83
PHOTOSYNTHESIS	3	thioredoxin M-type 4, chloroplast precursor (TRX-M4), glutathione synthetase (GSH2), chloroplast nucleoid DNA binding protein,	1.47-1.76
DETOXIFICATION	2	rhodanese-like domain protein	1.53-2.09

... continued ...

TABLE 2 Cont.

OTHER	20	steroid sulfotransferase, aldehyde oxidase, 12-oxophytodienoate reductase (OPR3), pescadillo - like protein, oxidoreductase, 2OG-Fe(II) oxygenase, nitrilase 1 like protein, hydrolase alpha/beta fold family, putative spermine/spermidine synthase, heme oxygenase 1 (HO1) gene, COP9 complex subunit, FUS4 FUSCA4, COP8, CSN4, acyltransferase family, fibrillarlin 2, oxidoreductase (din11), glutaredoxin protein, iron-sulfur cluster assembly complex protein, hypothetical protein, copper amine oxidase -related, glutaredoxin protein, aldo/keto reductase family, nodulin MtN3 family,	1.44-2.94
UNKNOWN	50	-	1.40-3.76
Down-Regulated = 53 genes – 20% of regulated genes total			
CELL STRUCTURE	3	histone H3, histone H2B, histone H1	0.58-0.60
CELL WALL	2	pectinesterase, N-acetylglucosaminyltransferase	0.38-0.47
DEFENSE RESPONSE	1	terpene synthase/cyclase	0.60
HORMONE RESPONSE	2	ethylene-response protein ETR1, GAST1-related protein induced by gibberellins	0.48-0.60
METABOLISM	1	sugar isomerase	0.55
PROTEIN BIOSYNTHESIS	1	Eukaryotic translation initiation factor 3 subunit 10	0.36
SIGNAL TRANSDUCTION	1	putative membrane protein	0.54
TRANSCRIPTION FACTOR	6	PHD finger transcription factor, lateral organ boundaries (LOB) domain protein 37, ANAC057; transcription factor, GATA zinc finger protein, WRKY family transcription factor	0.41-0.56
TRANSPORT	1	peptide transporter - like protein	0.23
ENERGY PATHWAYS	2	alkaline/neutral invertase, NAD-dependent epimerase/dehydratase	0.49-0.54
LIPID METABOLISM	2	lipoic acid synthase, 3-oxoacyl-[acyl-carrier-protein] synthase I precursor	0.41-0.55
PHOTOSYNTHESIS	10	ribulose bisphosphate carboxylase small chain 3b precursor, ribulose bisphosphate carboxylase small chain 2b precursor, plastocyanin, chlorophyll a-b binding protein 3C-like, light-harvesting chlorophyll a/b binding protein, protochlorophyllide reductase B	0.33-0.59
PROTEINASE INHIBITOR	7	proteinase inhibitor - tomato	0.42-0.48
OTHER	6	putative membrane-associated salt-inducible protein, hypothetical protein DDBDRAFT_0219654, germin-like protein, Tic62 protein, dopamine beta-monoxygenase, gda-1,	0.32-0.57
UNKNOWN	7		0.39-0.55

3.3 NEFID activates the signal transduction in tomato leaves

A total of 20 up-regulated genes were associated with signal transduction, included the induction of six protein kinases (phosphatidylinositol-4-phosphate 5-kinase, MAP3K-like protein kinase, mitogen-activated protein kinase kinase (MAPKK), leucine rich repeat protein family contains protein kinase domain, receptor-related serine/threonine kinase), five genes involved in the calcium signaling such as calcium-dependent protein kinase, calmodulin-binding protein, calmodulin and calcineurin-like phosphoesterase; five phosphatase encoding genes concerned in the dephosphorylation (protein phosphatase 2C (PP2C), tyrosine phosphatase) as well as a transmembrane protein and a transducin/WD-40 repeat protein involved in the GTP mediated signal cascade. In addition, five GTP binding protein (G protein - GTPase activating protein, ARF GTPase-activating domain, GTP-binding protein, Ras-related GTP-binding protein (ARA-4), putative ATP(GTP)-binding protein) and five transport-related genes (ATPase plasma membrane-type (proton pump), H⁺-transporting ATP synthase-related protein, SNF7 protein, putative UDP-galactose transporter, transporter - related low similarity to hexose transporter) were also up-regulated by the tested formulation.

3.4 NEFID induces hypersensitive reaction and defense response

One of the largest categories of genes regulated by NEFID treatment was the oxidative burst/hypersensitive response-related genes, with 17 genes up-regulated. A substantial proportion of these ESTs have predicted functions in cell rescue and defense processes such as peroxidase, glutathione peroxidase, copper/zinc superoxide dismutase (CSD2), copper/zinc superoxidase dismutase (CSD1), glyoxalase II, cytochrome P450, and glutathione transferase. This category comprises the genes that are responsible for the removal of ROSs.

These induced response support the important role of oxidative burst in activating defense genes.

3.5 NEFID activates PR protein activities

As a result of tomato plant elicitation by NEFID, an up-regulation of genes associated to a putatively known function can undergo post-translational modification and/or silencing which result in a phenotype different from the one inferred from transcriptomics. Thus, additional survey on key pathogenesis-related enzymes was conducted over a time course (0, 24, 48, 72, 96 and 120 hours after spraying) for the activities of peroxidase (POX), glucanase (GLU) and chitinase (CHI) and all were higher than the untreated control as early as 24 hours after spraying (Figure 3). Treatment of tomato plants with NEFID showed a significant ($p \leq 0.05$) increase of POX activity at 24, 48, 72, 96 and 120 HAS. At 48 HAS POX activity demonstrated an abrupt increase in values around fourfold higher than the control, after this peak the POX activity of plants treated with NEFID stayed in values around twofold higher than the control to 120 HAS (Figure 3a).

It was observed that treated plants were able to keep up GLU activity from 24 hours after spraying onward, whereas a tendency GLU activity decrease in water-sprayed control plants compared to the basal level was observed (Figure 3b). At 24-120 HAS interval, GLU activities on treated plants were significantly ($p \leq 0.05$) higher than that of control. At 96 HAS was verified an abrupt peak of GLU activity with values around threefold higher than the control.

Plants sprayed with NEFID, showed a remarkable peak of CHI activity at 24 HAS, twofold higher than the control and from 48-120 HAS and was similar to the control (Figure 3c).

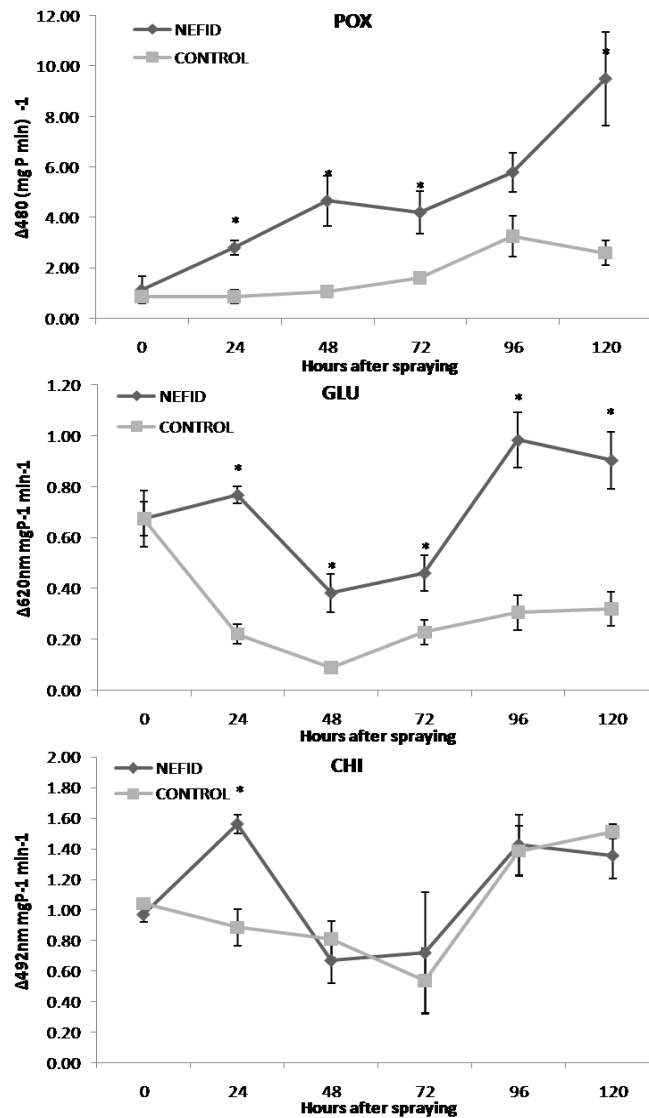


FIGURE 3 Activity of peroxidase (POX) (A), β -1,3-glucanase (GLU) (B), chitinase (CHI) (C) was induced after spraying the tomato seedlings with NEFID in comparison with the water-sprayed control. Enzymatic responses were evaluated 0, 24, 48, 72, 96, 120, hours after spraying. Error bars indicate standard deviations.

4 DISCUSSION

Gene expression profiling through the use of microarrays has been recognized as a powerful approach to obtain overall view on gene expression and physiological processes involved in response to a particular stimulus (Maleck et al., 2000; Schenk et al., 2000). In this study, to better understand underlying physiological events after a coffee leaf plant formulation treatment, we analyzed gene expression profiles of 11,000 tomato genes in tomato leaves at early stage after treatment (12 h) and identified a total of 268 genes that were differentially expressed, which might have been underestimated if the whole genome is considered in future studies since the total estimated tomato genome (35,000 genes) is three fold the number of ESTs used (Van der Hoeven et al., 2002).

From the assigned categories “signal transduction” represents the largest group (9% of up-regulated genes, 2% of down-regulated genes) and pieces of evidence link them (transmembrane ion balance and kinase cascade) to the upstream events underlying the observed defense responses regarding.

Transient changes in the ion permeability of the plasma membrane appear to be a common early event in defense signaling. Upon pathogen recognition, ion channels located in the plasma membrane appear to increase ion fluxes across the membrane and activate downstream defense responses (Wan et al., 2002). Maleck et al. (2000) identified some pump or ion channel genes that were up-regulated by defense-related treatments; for example, a plasma membrane H⁺-ATPase gene was up-regulated in a constitutive SAR mutants (*cim*) and up-regulated in systemic leaves expressing *avrRpt2* after challenging them with *P. syringae* pv. *tomato* DC3000. A rapid expression change as were observed for signal transduction, GTP binding protein and transport-related genes is a possible direct consequence of NEFID-mediated

plasma membrane ATPase (proton pump) activation, which may lead PR gene expression.

Another link between signal transduction and down-stream PR activation is related to the mitogen activated kinases (MAP3K and MAPKK) and calcium dependent kinases (calmodulin and calcium protein kinases) as well as the absence of evidence for salicylic or jasmonic acid/ethylene metabolisms suggesting for a kinase cascade signaling leading to a SAR-independent pathogenesis-related protein activation as previously shown for fusicoccin-mediated PR accumulation in tomato (Frick & Schaller, 2002).

Furthermore, the down-regulation of ethylene response protein and terpene synthase/cyclase (jasmonic acid biosynthesis) suggests that the observed disease resistance is not likely to have a consequence on fruit ripening. The constraint was pointed out by Jiang et al. (2009) studying post harvest disease resistance induction in cherry tomato. They found that in spite of the up-regulation of PRs in a JA-independent manner, an up-regulation of both jasmonic acid biosynthesis and ethylene receptors were up-regulated.

Another possible side effect related to induced resistance is the shift in the primary metabolism to provide backbone carbon for resistance proteins and related molecules such as glutamate decarboxylase and lipase (Shah, 2005) as well as a down-regulation of photosynthesis-related genes. The photosynthesis is not likely to be linked to the jasmonate-mediated inhibition of photosynthetic pigments and photosynthetic activity (Frick & Schaller, 2002) and measurements of photosynthesis at a later time point (8d after spray) showed no difference between NEFID-treated and control plants (Fig. 4), suggesting for a post-transcriptional regulation or silencing regulation of those genes.

4.1 Oxidative burst and hypersensitive reaction

The “oxidative burst/hypersensitive response” is among the largest group of genes with changed regulation after treatment with NEFID formulation (7% of up-regulated ones). Signaling compounds production such as reactive oxygen species (ROSs) causes activation of many downstream responses (Khan & Wilson, 1995). Increasing verification has suggested that ROSs play a role directly as antimicrobial compounds, and also as signaling molecules in plant defense. ROSs are important in activating defense gene expression in adjacent cells and the whole plant, probably in combination with other signaling molecules (Buchanan, 2000).

Because reactive oxygen species (ROS) can cause damage to proteins, lipids and DNA, ROS production and removal must be strictly controlled. Various ROS-scavenging systems, such as glutathione, superoxide dismutases, catalases and ascorbate peroxidases, maintain ROS homeostasis in different compartments of the plant cell (Mittler et al., 2004). These enzymes could restrict the ROS-dependent damage or finely tune ROS-dependent signal transduction (Torres et al., 2006). Differential regulation of these enzymes, in part mediated by SA, may contribute to increases in ROS and activation of defenses following infection (Dorey et al., 1998; Mittler et al., 1999; Klessig et al., 2000).

4.2 Defense-related

NEFID-induced expression changes of potential defense responses genes. Most of the up-regulated responses in defense-related transcripts have been reported as part of the salicylic acid dependent pathway (PR-1, lipid transfer protein TSW12, basic endochitinase, hevein-related protein precursor (PR-4), VIP2 protein, endo-1,4-beta-glucanase, disease resistance protein (NBS-LRR class), glucan endo-1,3-beta-glucosidase) except (PDF2.3) plant

defensin which is putatively a product of the jasmonate/ethylene pathway (Pieterse & van Loon, 1999). Conversely, the gene coding for terpene synthase/cyclase, which is involved in methyl jasmonate pathway biosynthesis (Martin et al., 2003), was down-regulated in NEFID-treated plants.

A marker gene of systemic acquired resistance (PR-1) is generally associated to the salicylic acid pathway but in this case no evidence of SA accumulation was found and future studies will monitor salicylic acid in different time points in order to determine if SA plays a role in upstream signaling of the observed PRs. However, the PR translation triggering has already been mentioned as SA-independent (Frick & Schaller, 2002).

Various are the roles of the observed PRs acting either directly on the pathogen or indirectly by creating physical barriers to the fungal infection process or upstream intrinsic PR signaling.

Most of the found PRs act directly on the disruption of the fungal/bacterial cell wall (endo-1,4-beta-glucanase, basic endochitinase and glucan endo-1,3-beta-glucosidase), or inhibiting the fungus germination due to the ribonuclease activity (hevein-like precursor) (Datta & Muthukrishnan, 1999; Caporale et al., 2004). And the assessment of the enzyme activities (glucanase and chitinase) (Fig. 3) confirms the translation of the protein as well as their long-lasting expression particularly glucanase.

Also found to be up-regulated was a defensin (PDF1.2), a gene putatively associated to the jasmonate-dependent pathway. Since no gene peculiar to this pathway was found, future experiments will determine the upstream signaling leading to the expression of this protein (Pieterse & van Loon, 1999).

In an indirect way, the lipid transfer protein TSW12 is referred to as cuticle formation, an indirect protection against pathogen invasion as well as drought tolerance (Torres-Schumann et al., 1992; Molina & Garcia-Olmedo,

1997). As early events of the defense signaling, the disease resistance protein (NBS-LRR class) plays a role in fast signal transduction (Belkhadir, 2004).

4.3 Diverse possible roles of the NEFID-mediated changes

Apart from the PRs, the cytochrome P450, found to be up-regulated, has been reported in the production of antimicrobial substances (Jiang et al., 2009) and may play an additional role in the defense against pathogens.

Since plants haven't been challenged with any pathogen, which possibly fine tunes the plant metabolism for the defense responses (Guzzo et al., 2009), broad responses were observed such as the possible up-regulation of genes involved in stress alleviation in regard to reactive oxygen species (glutathione S-transferase, peroxidase) or temperature (heat shock protein).

4.4 Traits with unknown role on defense responses

The “transcription factor” represents the largest number of genes in both up and down regulation (8% of up-regulated genes, 11% of down-regulated genes) but the direct relationship on disease resistance needs further evidence.

Similar to the mentioned category, the role of other categories not detailed in this study will be the focus of future experiments.

In previous works, the effectiveness of the rust infected coffee leaf formulation has been demonstrated in the control of either fungal or bacterial diseases in coffee or cotton (Santos et al., 2007; Ishida et al., 2007), previous work has also demonstrated its potential in the control of bacterial spot as well as the commercial elicitor acibenzolar-S-methyl (data not presented) and in the study we chose not to inoculate plants to have an insight of the broad range of possible targets of the NEFID-mediated disease resistance and used tomato as the model plant. Potentially responses to bacterial (glucanase/lisozyme and

defensin) and fungal (chitinase, glucanase and hevein) have been identified, not impossible that other pathogens may be affected by the afore-mentioned PRs such as nematodes and virus.

Nematode eggs are made up of chitin and chitinase has already been implicated in disruption of egg shells leading to a reduction in hatching (Qiu et al., 1997). A more complicated case is the induction of resistance against virus. A piece of evidence suggests that chaperone interact with the movement protein of the virus hindering its cell-to-cell movement (Von Bargen et al., 2001).

A fungal cell derivative (chitosan), such as the one likely to be present in the used infected leaf extract was found to induce the expression of DNAJ-related chaperone, also found as up-regulated in our studies, similar to the one found in the control of TSWV in tomato, tobacco and *Arabidopsis* (Zhang et al., 2007). The same elicitor has proven to control the root knot nematode *Meloidogyne incognita*, showing a reduction in number of eggs and fertile females and the mode of action was the activation of PRs, notably chitinase (Zinov'eva et al., 2001).

Finally, the tested plant disease elicitor, NEFID, has shown to induce multiple plant defense responses with the potential to be used against multiple pathogens. The induction does not interfere in the photosynthesis and the pathogenesis-related proteins are not likely to suffer post-tranlational regulation since the glucanase, peroxidase and chitinase activities were found to be over-expressed as early as 24h after spray and eventually last for at least five days (glucanase and peroxidase) at high levels, suggesting for a long lasting effect of elicitation.

5 REFERENCES

- BARGEN, S. von; SALCHERT, K.; PAAPE, M.; PIECHULLA, B.; KELLMANN, J. W. Interactions between the tomato spotted wilt virus movement protein and plant proteins showing homologies to myosin, kinesin and DnaJlike chaperones. **Plant Physiology and Biochemistry**, Paris, v. 39, n. 12, p. 1083-1093, Dec. 2001.
- BARGUIL, B. M.; RESENDE, M. L. V.; RESENDE, R. S.; BEZERRA JUNIOR, J. E. A.; SALGADO, S. L. Effect of extracts from citric biomass, rusted coffee leaves and coffee berry husks on *Phoma costaricensis* of coffee plants. **Fitopatologia Brasileira**, Fortaleza, v. 30, n. 5, p. 535-537, set./out. 2005.
- BARONE, A.; CHIUSANO, M. L.; ERCOLANO, M. R.; GIULIANO, G.; GRANDILLO, S.; FRUSCIANTE, L. Structural and functional genomics of tomato. **International Journal of Plant Genomics**, Chicago, v. 2008, p. 1-12, Jan. 2008.
- BELKHADIR, Y.; SUBRAMANIAM, R.; DANGL, J. L. Plant disease resistance protein signaling: NBS-LRR proteins and their partners. **Current Opinion in Plant Biology**, London, v. 7, n. 4, p. 391-399, Aug. 2004.
- BLANCO, F.; SALINAS, P.; CECCHINI, N. M.; JORDANA, X.; HUMMELEN, P. van; ALVAREZ, M. E.; HOLUIQUE, L. Early genomic responses to salicylic acid in Arabidopsis. **Plant Molecular Biology**, Dordrecht, v. 70, n. 1/2, p. 79-102, May 2009.
- BRADFORD, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, San Diego, v. 72, n. 1/2, p. 248-254, Jan. 1976.
- BUCHANAN, B. B.; GRUISSEM, W.; JONES, R. L. **Biochemistry and molecular biology of plants**. Rockville: American Society of Plant Physiologists, 2000. 1367 p.
- CAPORALE, C.; DI BERARDINO, I.; LEONARDI, L.; BERTINI, L.; CASCONI, A.; BUONOCORE, V.; CARUSO, C. Wheat pathogenesis-related proteins of class 4 have ribonuclease activity. **FEBS Letters**, Amsterdam, v. 575, n. 1/3, p. 71-76, Sept. 2004.

CAVALCANTI, F. R.; RESENDE, M. L. V.; CARVALHO, C. P.; SILVEIRA, J. A.; OLIVEIRA, J. T. An aqueous suspension of *Crinipellis pernicios* mycelium activates tomato defence responses against *Xanthomonas vesicatoria*. **Crop Protection**, Oxford, v. 26, n. 5, p. 729-738, May 2007.

DATTA, K.; MUTHUKRISHNAN, S. **Pathogenesis-related proteins in plants**. Boca Raton: CRC, 1999. 357 p.

DOREY, S.; BAILLIEUL, F.; SAINDRENAN, P.; FRITIG, B.; KAUFFMANN, S. Tobacco class I and II catalases are differentially expressed during elicitor induced hypersensitive cell death and localized acquired resistance. **Molecular Plant-Microbe Interaction**, Saint Paul, v. 11, n. 11, p. 1102-1109, Nov. 1998.

FRICK, U. B.; SCHALLER, A. cDNA microarray analysis of fusicoccin-induced changes in gene expression in tomato plants. **Planta**, New York, v. 216, n. 1, p. 83-94, Nov. 2002.

GORE, J. P.; GARRO, L. W. *Xanthomonas campestris* pv. *vesicatoria* from bell pepper and tomato in Barbados undergoes changes in race structure, virulence and sensitivity to chemical control agents. **Journal of Phytopathology**, Berlin, v. 147, n. 7/8, p. 397-402, Aug. 1999.

GUZZO, S. D.; HARAKAVA, R.; TSAI, S. M. Identification of coffee genes expressed during systemic acquired resistance and incompatible interaction with *Hemileia vastatrix*. **Journal of Phytopathology**, Berlin, v. 99, n. 1, p. 1538, Jan. 2009.

HOEVEN, R. van der; RONNING, C.; GIOVANNONI, J.; MARTIN, G.; TANKSLEY, S. Deductions about the number, organization, and evolution of genes in the tomato genome based on analysis of a large expressed sequence tag collection and selective genomic sequencing. **Plant Cell**, Rockville, v. 14, n. 7, p. 1441-1456, July 2002.

ISHIDA, A. K. N.; SOUZA, R. M.; ZACARONI, A. B.; RIBEIRO JÚNIOR, P. M.; AMARAL, D. R.; RESENDE, M. L. V. Extrato vegetal e acibenzolar-S-metil (ASM) na indução de resposta de defesa do algodoeiro contra *Xanthomonas axonopodis* pv. *malvacearum*. In: CONGRESSO BRASILEIRO DE FITOPATOLOGIA, 40., 2007, Maringá. **Anais...** Lavras: UFLA, 2007. p. S262.

JIANG, F.; ZHENG, X.; CHEN, J. Microarray analysis of gene expression profile induced by the biocontrol yeast *Cryptococcus laurentii* in cherry tomato fruit. **Gene**, Amsterdam, v. 430, n. 1/2, p. 12-16, Feb. 2009.

JONES, J. B.; WOLTZ, S. S.; JONES, J. P.; PORTIER, K. L. Population dynamics of *Xanthomonas campestris* pv. *vesicatoria* on tomato leaflets treated with copper bactericides. **Phytopathology**, Saint Paul, v. 81, n. 7, p. 714-719, July 1991.

KHAN, A. U.; WILSON, T. Reactive oxygen species as cellular messengers. **Chemistry & Biology**, London, v. 2, n. 7, p. 437-445, July 1995.

KLESSIG, D. F.; DURNER, J.; NOAD, R.; NAVARRE, D. A.; WENDEHENNE, D.; KUMAR, D.; ZHOU, J. M.; SHAH, J.; ZHANG, S.; KACHROO, P.; TRIFA, Y.; PONTIER, D.; LAM, E.; SILVA, H. Nitric oxide and salicylic acid signaling in plant defense. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, DC, v. 97, n. 16, p. 8849-8855, Aug. 2000.

LOUWS, F. J.; WILSON, M.; CAMBELL, H. L.; CUPPELS, D. A.; JONES, J. B.; SHOEMAKER, P. B.; SAHIN, F.; MILLER, S. A. Field control of bacterial spot and bacterial speck of tomato using a plant activator. **Plant Disease**, Saint Paul, v. 85, n. 5, p. 481-488, May 2001.

MALECK, K.; LEVINE, A.; EULGEM, T.; MORGAN, A.; SCHMID, J.; LAWTON, K. A.; DANGL, J. L.; DIETRICH, R. A. The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. **Nature Genetics**, New York, v. 26, n. 4, p. 403-410, Dec. 2000.

MARTIN, D.; GERSHENZON, J.; BOHLMANN, J. Induction of volatile terpene biosynthesis and diurnal emission by methyl jasmonate in foliage of Norway spruce. **Plant Physiology**, Rockville, v. 132, n. 3, p. 1586-1599, July 2003.

MITTLER, R.; HERR, E. H.; ORVAR, B. L.; CAMP, W. van; WILKENS, H.; INZE, D.; ELLIS, B. E. Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hyperresponsive to pathogen infection. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, DC, v. 96, n. 24, p. 14165-14170, Nov. 1999.

- MITTLER, R.; VANDERAUWERA, S.; GOLLERY, M.; BREUSEGEM, F. van. Reactive oxygen gene network of plants. **Trends in Plant Science**, London, v. 9, n. 10, p. 490-498, Oct. 2004.
- MOLINA, A.; GARCIA-OLMEDO, F. Enhanced tolerance to bacterial pathogens caused by the transgenic expression of barley lipid transfer protein LTP2. **Plant Journal**, Oxford, v. 12, n. 3, p. 669-675, Sept. 1997.
- MOORE, S.; PAYTON, P.; WRIGHT, P.; TANKSLEY, S.; GIOVANNONI, J. Utilization of tomato microarrays for comparative gene expression analysis in the Solanaceae. **Journal of Experimental Botany**, Oxford, v. 56, n. 421, p. 2885-2895, Nov. 2005.
- PIETERSE, C. M. J.; LOON, L. C. van. Salicylic acid-independent plant defence pathways. **Trends in Plant Science**, London, v. 4, n. 2, p. 52-58, Feb. 1999.
- QIU, J.; HALLMANN, J.; KOKALIS-BURELLE, N. Activity and differential induction of chitinase isozymes in soybean cultivars resistant or susceptible to root-knot nematodes. **Journal of Nematology**, Lakeland, v. 24, n. 4, p. 523-530, Dec. 1997.
- RESENDE, M. L. V.; ARAUJO, D. V.; COSTA, J. C. B.; DEUNER, C. C.; FERREIRA, J. B.; MUNIZ, M. F. S.; REIS, S. N.; SANTOS, F. S.; CAVALCANTI, L. S.; NOJOSA, G. B. A. Produtos comerciais a base de bioindutores de resistência em plantas. **Revisão Anual de Patologia de Plantas**, Passo Fundo, v. 14, n. 1, p. 361-380, jan. 2006.
- SANTOS, F. S.; SOUZA, P. E.; RESENDE, M. L. V.; POZZA, E. A.; MIRANDA, J. C.; RIBEIRO JÚNIOR, P. M.; MANERBA, F. C. Efeito de extratos vegetais no progresso de doenças foliares do cafeeiro orgânico. **Fitopatologia Brasileira**, Lavras, v. 32, n. 1, p. 59-63, fev. 2007.
- SCHENK, P. M.; KAZAN, K.; WILSON, I.; ANDERSON, J. P.; RICHMOND, T.; SOMERVILLE, S. C.; MANNERS, J. M. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, DC, v. 97, n. 21, p. 11655-11660, Oct. 2000.
- SHAH, J. Lipids, lipases, and lipid-modifying enzymes in plant disease resistance. **Annual Review of Phytopathology**, Palo Alto, v. 43, n. 1, p. 229-260, Jan. 2005.

SOUZA, J. L.; RESENDE, P. **Manual de horticultura orgânica**. Viçosa, MG: Aprenda Fácil, 2003. 564 p.

SOUZA, M. F. M.; RODRIGUES, R.; AMARAL JUNIOR, A. T.; SUDRE, C. P. Resistance to *Xanthomonas* spp. in tomato: diallel analysis and gene effects estimative in a breeding programme carried out in Brazil. **Journal of Phytopathology**, Oxford, v. 156, n. 11/12, p. 660-667, Dec. 2008.

TORRES, M. A.; JONES, J. D.; DANGL, J. L. Reactive oxygen species signaling in response to pathogens. **Plant Physiology**, Rockville, v. 141, n. 2, p. 373-378, June 2006.

TORRES-SCHUMANN, S.; GODOY, J. A.; PINTOR-TORO, J. A. A probable lipid transfer protein gene is induced by NaCl in stems of tomato plants. **Plant Molecular Biology**, Dordrecht, v. 18, n. 4, p. 749-757, Feb. 1992.

UNIVERSIDADE FEDERAL DE LAVRAS. RESENDE, M. L. V.; CAVALCANTI, F. R.; SANTOS, F. S.; RIBEIRO JUNIOR, P. M.; AMARAL, D. R. **Formulação para indução de resistência em plantas, a base de extrato vegetal obtido de folhas do cafeeiro**. BR n. INPI 0000220604167501. 2 ago. 2006.

URBANEK, H.; KUZNIAK-GEBAROWSKA, E.; HERKA, H. Elicitation of defence responses in bean leaves by *Botrytis cinerea* polygalacturonase. **Acta Physiologiae Plantarum**, Copenhagen, v. 13, n. 1, p. 43-50, Jan. 1991.

WAN, J.; DUNNING, F. M.; BENT, A. F. Probing plant-pathogen interactions and downstream defense signaling using DNA microarrays. **Functional and Integrative Genomics**, Berlin, v. 2, n. 6, p. 259-273, Nov. 2002.

WANG, J. F.; JONES, J. B.; SCOTT, J. W.; STALL, R. E. Several genes in *Lycopersicon esculentum* control hypersensitivity to *Xanthomonas campestris* pv. *vesicatoria*. **Phytopathology**, Saint Paul, v. 84, n. 7, p. 702-706, May 1994.

ZHANG, H.; KIM, M. S.; KRISHNAMACHARI, V.; PARE, P. W. Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in Arabidopsis. **Planta**, New York, v. 226, n. 4, p. 839-851, Sept. 2007.

ZINOV'eva, S. V.; PEREKHOD, E. A.; IL'INA, A. V.; UDALOVA, Z. H. V.;
GERASIMOVA, N. G.; VASYUKOVA, N. I.; OZERETSKOVSKAYA, O. L.;
SONIN, M. D. Proteins in plants infested with the root-knot nematode
Meloidogyne incognita (Kofoid et White, 1919) Chitwood 1949. **Doklady**
Biological Sciences, Heidelberg, v. 379, n. 1/6, p. 393-395, July 2001.

GENERAL CONCLUSIONS

Tomato plants treated with a natural compost of coffee leaf (NEFID) demonstrated 35% of reduction to the bacterial spot severity, a significantly reduction (61%) was obtained by ASM-treated plants.

The severity reduction of the bacterial spot disease appears to be based on a premature β -1,3-glucanase and PR-1 gene expression increase (12 hours after spraying) followed by an early response in a chitinase, glucanase and polyphenol oxidase activity (24 hours after spraying) and a earlier high lignin deposition on leaf tissue.

A natural compost of coffee leaf (NEFID) has the potential to activate defense related genes and they can be monitored using microarray technology.

A total of 268 genes had changed regulation with a majority of up-regulated ones which encoded mainly signal transduction, defense –related, oxidative burst and transcription factor, when tomato plants are sprayed with the NEFID.

Since no evidence for salicylic acid or jasmonic acid was found but mitogen activated proteins (MAP3K and MAPKK) as well as calcium dependent (calmodulin and phosphatidylinositol) signaling molecules were found, a SA-independent PR accumulation is likely to occur.

The PRs chitinase, glucanase and hevein-like, with direct reported activity on pathogens, were up-regulated with the application of NEFID on tomato plants, and they are not likely to suffer post-translational regulation since the corresponding enzyme activities were over-expressed as early as 24h after treatment and eventually remained as such for up to five days onward (glucanase and peroxidase).

The studied plant formulation represent a potential broad spectrum disease control.

ANEXES

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TABLE 1 NEFID-responsive tomato genes identified by microarray approaches.

ID	Gene Annotation	Average of ratios	Pvalue
Up-Regulated			
NUCLEIC ACID METABOLISM			
M84744	Tomato phytoene synthetase mRNA, complete cds	1.49	0.01
SGN-U212772	arabidopsis/peptide: At3g62290.1 68410.m06466 ADP-ribosylation factor identical to GP:166586 ADP-ribosylation factor {Arabidopsis thaliana}; ADP-ribosylation factor 1 - Arabidopsis thaliana, PIR:S28875 (evalue: 3e-100, score=361.3) genbank/nr: gi 1703380 sp P51823 ARF_ORYSA ADP-ribosylation factor gi 25294170 pir T52341 ADP-ribosylation factor [imported] - rice gi 1132483 dbj BAA04607.1 ADP-ribosylation factor [Oryza sativa (japonica cultivar-group)] gi 13646976 dbj BAB41081.1 ADP-ribosylation factor [Triticum aestivum] gi 23304413 emb CAD48129.2 ADP-ribosylation factor 1-like protein [Hordeum vulgare subsp. vulgare] (evalue: 8e-100, score=365.2)	1.79	0.04
SGN-U214340	arabidopsis/peptide: At3g19130.1 68410.m02193 RNA-binding protein, putative similar to RNA Binding Protein 47 [Nicotiana glumbaginifolia] GI:9663769, DNA binding protein ACBF GB:AAC49850 from [Nicotiana tabacum]; contains InterPro entry IPR000504: RNA-binding region RNP-1 (RNA recognition motif) (RRM) (evalue: 2e-125, score=446) genbank/nr: gi 7489196 pir T01932 RNA binding protein homolog - common tobacco (fragment) gi 2708532 gb AAB92518.1 putative RNA binding protein [Nicotiana tabacum] (evalue: 9e-164, score=578.9)	1.44	0.08
SGN-U215885	arabidopsis/peptide: At1g47510.1 68408.m04833 endonuclease/exonuclease/phosphatase family similar to SP P32019 Type II inositol-1,4,5-trisphosphate 5-phosphatase precursor (EC 3.1.3.56) {Homo sapiens}; contains Pfam profile PF03372: Endonuclease/Exonuclease/phosphatase family (evalue: 2e-108, score=389) genbank/nr: gi 37718787 gb AAR01658.1 putative inositol polyphosphate 5-phosphatase [Oryza sativa (japonica cultivar-group)] (evalue: 7e-112, score=406)	1.83	0.03
SGN-U227378	arabidopsis/peptide: At3g45630.1 68410.m04565 RNA recognition motif (RRM) - containing protein similar to SP P34909 General negative regulator of transcription subunit 4 {Saccharomyces cerevisiae}; contains InterPro entry IPR000504: RNA-binding region RNP-1 (RNA recognition motif) (RRM) (evalue: 2.5e-96, score=348.2) genbank/nr: gi 15231193 ref NP_190149.1 RNA recognition motif (RRM)-containing protein [Arabidopsis thaliana] gi 11357925 pir T47503 hypothetical protein F9K21.210 - Arabidopsis thaliana gi 6996266 emb CAB75492.1 putative protein [Arabidopsis thaliana] (evalue: 8.9e-95, score=348.2)	2.05	0.04

... continued...

TABLE 1 Cont.

CELL STRUCTURE			
SGN-U213866	arabidopsis/peptide: At1g51060.1 68408.m05267 histone H2A, putative similar to histone H2A GI:7595337 from Arabidopsis thaliana, Triticum aestivum GI:536892, Picea abies SP P35063; contains Pfam profile PF00125 Core histone H2A/H2B/H3/H4 (evalue: 6.1e-46, score=181) genbank/nr: gi 15223708 ref NP_175517.1 histone H2A, putative [Arabidopsis thaliana] gi 25294262 pir G96547 probable histone H2A [imported] - Arabidopsis thaliana gi 12320785 gb AAG50540.1 histone H2A, putative [Arabidopsis thaliana] gi 13877851 gb AAK44003.1 putative histone H2A protein [Arabidopsis thaliana] gi 17065594 gb AAL33777.1 putative histone H2A protein [Arabidopsis thaliana] (evalue: 2.2e-44, score=181)	1.51	0.04
SGN-U214142	arabidopsis/peptide: At5g56600.1 68412.m06396 profilin 5 (evalue: 2.1e-48, score=189.1) genbank/nr: gi 16555787 emb CAD10377.1 profilin [Lycopersicon esculentum] (evalue: 1.4e-61, score=238)	2.14	0.04
SGN-U215974	arabidopsis/peptide: At5g56180.1 68412.m06343 actin-related protein 8B (ARP8) protein annotation temporarily based on supporting cDNA gi 21427470 gb AF507916.1 (evalue: 0, score=634) genbank/nr: gi 30696705 ref NP_568836.2 actin-related protein 8B (ARP8) protein [Arabidopsis thaliana] gi 9758638 dbj BAB09300.1 contains similarity to actin~gene_id:MDA7.24 [Arabidopsis thaliana] gi 21427471 gb AAM53248.1 actin-related protein 8B [Arabidopsis thaliana] gi 21489926 tpg DAA00031.1 TPA: actin-related protein 8B; AtARP8B [Arabidopsis thaliana] (evalue: 3e-180, score=634)	1.68	0.07
SGN-U217962	arabidopsis/peptide: At1g51060.1 68408.m05267 histone H2A, putative similar to histone H2A GI:7595337 from Arabidopsis thaliana, Triticum aestivum GI:536892, Picea abies SP P35063; contains Pfam profile PF00125 Core histone H2A/H2B/H3/H4 (evalue: 4.1e-32, score=134.8) genbank/nr: gi 15223708 ref NP_175517.1 histone H2A, putative [Arabidopsis thaliana] gi 25294262 pir G96547 probable histone H2A [imported] - Arabidopsis thaliana gi 12320785 gb AAG50540.1 histone H2A, putative [Arabidopsis thaliana] gi 13877851 gb AAK44003.1 putative histone H2A protein [Arabidopsis thaliana] gi 17065594 gb AAL33777.1 putative histone H2A protein [Arabidopsis thaliana] (evalue: 1.4e-30, score=134.8)	1.56	0.01
SGN-U219052	arabidopsis/peptide: At1g60430.1 68408.m06240 expressed protein similar to putative actin polymerisation complex protein GI:4539247 from [Schizosaccharomyces pombe] (evalue: 5e-85, score=311.2) genbank/nr: gi 21536845 gb AAM61177.1 Contains similarity to 21 KD subunit of the Arp2/3 protein complex (ARC21) [Arabidopsis thaliana] (evalue: 1.9e-83, score=311.2)	1.62	0.08
CELL WALL			

... continued ...

TABLE 1 Cont.

SGN-U213455	arabidopsis/peptide: At5g57560.1 68412.m06509 xyloglucan endotransglycosylase (TCH4) identical to xyloglucan endotransglycosylase TCH4 protein GI:886116 (evaluate: 1e-101, score=366.7) genbank/nr: gi 1076604 pir S49812 xyloglucan endo-1,4-beta-D-glucanase (EC 3.2.1.-) precursor (clone tXET-B1) - tomato gi 577066 emb CAA58003.1 xyloglucan endo-transglycosylase [Lycopersicon esculentum] (evaluate: 7e-153, score=542.3)	2.18	0.03
SGN-U218121	arabidopsis/peptide: At1g64390.1 68408.m06687 glycosyl hydrolase family 9 (endo-1,4-beta-glucanase) similar to endo-beta-1,4-glucanase GI:4972236 from [Fragaria x ananassa] (Plant Mol. Biol. 40, 323-332 (1999)) (evaluate: 0, score=803.9) genbank/nr: gi 4165132 gb AAD08699.1 endo-beta-1,4-D-glucanase [Lycopersicon esculentum] (evaluate: 0, score=995.3)	1.42	0.02
SGN-U214839	arabidopsis/peptide: At5g49720.1 68412.m05554 glycosyl hydrolase family 9 (endo-1,4-beta-glucanase) similar to endo-1,4-beta-D-glucanase; cellulase GI:5689613 from [Brassica napus] (evaluate: 0, score=950.7) genbank/nr: gi 7488979 pir T07612 cellulase (EC 3.2.1.4) Cel3, membrane-anchored - tomato gi 2065531 gb AAC49704.1 endo-1,4-beta-glucanase [Lycopersicon esculentum] (evaluate: 0, score=1072.8)	1.71	0.01
SGN-U215711	arabidopsis/peptide: At2g39700.1 68409.m04413 expansin, putative (EXP4) similar to alpha-expansin 6 precursor GI:16923359 from [Cucumis sativus]; alpha-expansin gene family, PMID:11641069 (evaluate: 7e-124, score=440.7) genbank/nr: gi 7488994 pir T07630 expansin 1 - tomato gi 2062421 gb AAC63088.1 expansin [Lycopersicon esculentum] gi 33334359 gb AAQ12264.1 expansin 1 protein; LeExp1 [Lycopersicon esculentum] (evaluate: 3e-152, score=540)	1.82	0.08
SGN-U216932	arabidopsis/peptide: At3g53140.1 68410.m05423 O-diphenol-O-methyl transferase, putative similar to GI:6688808 [Medicago sativa subsp. x varia], caffeic acid O-methyltransferase (homt1), Populus kitakamiensis, EMBL:PKHOMT1A (evaluate: 7e-140, score=493.8) genbank/nr: gi 15231756 ref NP_190882.1 O-diphenol-O-methyl transferase, putative [Arabidopsis thaliana] gi 11279303 pir T46160 caffeic acid O-methyltransferase-like protein - Arabidopsis thaliana gi 6630734 emb CAB64217.1 caffeic acid O-methyltransferase-like protein [Arabidopsis thaliana] gi 14194165 gb AAK56277.1 AT3g53140/T4D2_70 [Arabidopsis thaliana] gi 22137206 gb AAM91448.1 AT3g53140/T4D2_70 [Arabidopsis thaliana] (evaluate: 3e-138, score=493.8)	1.46	0.04
SGN-U218042	arabidopsis/peptide: At1g11545.1 68408.m01194 xyloglucan endotransglycosylase, putative similar to endo-xyloglucan transferase GI:2244732 from [Gossypium hirsutum] (evaluate: 4.7e-85, score=243.4) genbank/nr: gi 18391291 ref NP_563892.1 xyloglucan endotransglycosylase, putative [Arabidopsis thaliana] (evaluate: 1.7e-83, score=243.4)	1.85	0.06

... continued ...

TABLE 1 Cont.

SGN-U220366	arabidopsis/peptide: At3g58790.1 68410.m06057 glycosyltransferase family 8 contains Pfam profile: PF01501 glycosyl transferase family 8; general stress protein gspA, <i>Bacillus subtilis</i> , PIR:S16423 (evaluate: 2e-162, score=569.3) genbank/nr: gi 22331857 ref NP_191438.2 glycosyltransferase family 8 [<i>Arabidopsis thaliana</i>] gi 20466464 gb AAM20549.1 putative protein [<i>Arabidopsis thaliana</i>] gi 22136432 gb AAM91294.1 putative protein [<i>Arabidopsis thaliana</i>] (evaluate: 9e-161, score=569.3)	1.55	0.09
SGN-U220698	arabidopsis/peptide: At4g19900.1 68411.m02661 glycosyltransferase-related contains Pfam profiles PF01535: PPR repeat, PF04572: Alpha 1,4-glycosyltransferase conserved region, PF04488: Glycosyltransferase sugar-binding region containing DXD motif; several hypothetical proteins - <i>Arabidopsis thaliana</i> (evaluate: 5.2e-27, score=117.9) genbank/nr: gi 34899808 ref NP_911250.1 OJ1092_A07.13 [<i>Oryza sativa</i> (japonica cultivar-group)] gi 27817893 dbj BAC55659.1 unknown protein [<i>Oryza sativa</i> (japonica cultivar-group)] (evaluate: 5.6e-27, score=122.9)	1.55	0.01
DEFENSE RESPONSE			
SGN-U212883	arabidopsis/peptide: At3g12500.1 68410.m01395 glycosyl hydrolase family 19 (basic endochitinase) identical to basic endochitinase precursor SP:P19171 from [<i>Arabidopsis thaliana</i>] (evaluate: 1e-123, score=439.9) genbank/nr: gi 544011 sp Q05538 CHIC_LYCES Basic 30 kDa endochitinase precursor gi 487033 pir S37344 chitinase (EC 3.2.1.14) chi9 precursor - tomato gi 19191 emb CAA78845.1 chitinase [<i>Lycopersicon esculentum</i>] (evaluate: 7e-169, score=595.5)	1.65	0.08
SGN-U212922	arabidopsis/peptide: At2g14580.1 68409.m01475 pathogenesis-related protein, putative similar to SP P33154 Pathogenesis-related protein 1 precursor (PR-1) { <i>Arabidopsis thaliana</i> }; contains Pfam profile PF00188: SCP-like extracellular protein (evaluate: 1.8e-49, score=192.6) genbank/nr: gi 548587 sp P04284 PR06_LYCES Pathogenesis-related leaf protein 6 precursor (P6) (Ethylene-induced protein P1) (P14) (P14A) (PR protein) gi 2144919 pir VCTO14 pathogenesis-related protein P6 precursor - tomato gi 19285 emb CAA48672.1 P1(p14) protein [<i>Lycopersicon esculentum</i>] gi 170490 gb AAA03616.1 pathogenesis-related protein P6 gi 2529165 emb CAA70042.1 PR protein [<i>Lycopersicon esculentum</i>] (evaluate: 4.7e-94, score=345.9)	1.58	0.02
SGN-U212923	arabidopsis/peptide: At4g33720.1 68411.m04354 pathogenesis-related protein, putative similar to SP P33154 Pathogenesis-related protein 1 precursor (PR-1) { <i>Arabidopsis thaliana</i> }; contains Pfam profile PF00188: SCP-like extracellular protein (evaluate: 3.8e-51, score=198.7) genbank/nr: gi 548586 sp Q04108 PR04_LYCES Pathogenesis-related leaf protein 4 precursor (P4) gi 2119761 pir S26238 pathogenesis-related protein isoform P4 precursor - tomato gi 170488 gb AAA03615.1 pathogenesis-related protein P4 gi 3660529 emb CAA09671.1 pathogenesis-related protein PR1a (P4) [<i>Lycopersicon esculentum</i>] (evaluate: 3.3e-94, score=347.1)	1.84	0.01

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TABLE 1 Cont.

SGN-U212943	arabidopsis/peptide: At3g46570.1 68410.m04684 glycosyl hydrolase family 17 similar to glucan endo-1,3-beta-glucosidase precursor SP:P52409 from [<i>Triticum aestivum</i>] (evalue: 1.5e-20, score=96.29) genbank/nr: gi 13548679 dbj BAB40807.1 endo-1,3-beta-glucanase-like protein [<i>Pyrus pyrifolia</i>] (evalue: 6.1e-23, score=109.4)	1.61	0.02
SGN-U213613	arabidopsis/peptide: At5g43580.1 68412.m04803 hypothetical protein (evalue: 3.6e-08, score=54.3) genbank/nr: gi 124192 sp P20076 IER1_LYCES Ethylene-responsive proteinase inhibitor I precursor gi 82085 pir A32067 ethylene-responsive proproteinase inhibitor I precursor - tomato gi 623594 gb AAA60745.1 proteinase inhibitor I (evalue: 1e-09, score=64.31)	2.89	0.09
SGN-U214103	arabidopsis/peptide: At5g61240.1 68412.m06950 leucine rich repeat protein family contains leucine rich-repeat (LRR) domains Pfam:PF00560, INTERPRO:IPR001611; contains similarity to Hcr2-0B [<i>Lycopersicon esculentum</i>] gi 3894387 gb AAC78593 (evalue: 1e-131, score=466.8) genbank/nr: gi 14626935 gb AAK70805.1 leucine-rich repeat resistance protein-like protein [<i>Gossypium hirsutum</i>] (evalue: 2e-140, score=501.5)	1.48	0.03
SGN-U214589	arabidopsis/peptide: At2g02130.1 68409.m00129 plant defensin protein, putative (PDF2.3) plant defensin protein family member, personal communication, Bart Thomma (Bart.Thomma@agr.kuleuven.ac.be) (evalue: 5.8e-09, score=57.38) genbank/nr: gi 4753797 emb CAB42006.1 gamma-thionin [<i>Lycopersicon esculentum</i>] (evalue: 2.5e-15, score=83.57)	1.58	0.09
SGN-U214985	arabidopsis/peptide: At3g04720.1 68410.m00468 hevein-related protein precursor (PR-4) identical to hevein-like protein precursor GB:P43082 [<i>Arabidopsis thaliana</i>], similar to wound-induced protein (WIN2) precursor GB:P09762 [<i>Solanum tuberosum</i>]; Pfam HMM hit: chitin_binding proteins (evalue: 7.3e-51, score=196.8) genbank/nr: gi 400851 sp P32045 PRP2_LYCES Pathogenesis-related protein P2 precursor gi 100232 pir S23801 pathogenesis-related protein P2 precursor - tomato gi 19976 emb CAA41439.1 pathogenesis-related protein P2 [<i>Lycopersicon esculentum</i>] (evalue: 1e-82, score=307.8)	1.68	0.06
SGN-U215247	arabidopsis/peptide: At5g59710.1 68412.m06775 VIP2 protein annotation temporarily based on supporting cDNA gi 12006938 gb AF295433.1 AF295433 (evalue: 2.7e-72, score=269.2) genbank/nr: gi 25406997 pir B86212 protein F24B9.20 [imported] - <i>Arabidopsis thaliana</i> gi 8439898 gb AAF75084.1 F24B9.20 [<i>Arabidopsis thaliana</i>] (evalue: 3.5e-79, score=297.4)	1.86	0.08
SGN-U224778	arabidopsis/peptide: At3g12500.1 68410.m01395 glycosyl hydrolase family 19 (basic endochitinase) identical to basic endochitinase precursor SP:P19171 from [<i>Arabidopsis thaliana</i>] (evalue: 1.9e-10, score=63.16) genbank/nr: gi 46396546 sp Q9S8M0 LECT_SOLTU Chitin-binding lectin 1 precursor (PL-I) (evalue: 8.2e-42, score=172.6)	2.03	0.06

... continued ...

TABLE 1 Cont.

SGN-U225938	arabidopsis/peptide: At2g25470.1 68409.m02764 leucine rich repeat protein family contains leucine rich-repeat (LRR) domains Pfam:PF00560, INTERPRO:IPR001611; contains similarity to disease resistance protein [<i>Lycopersicon esculentum</i>] gi 3894383 gb AAC78591 (evaluate: 2.5e-10, score=62.77) genbank/nr: gi 44888781 gb AAS48162.1 LRR protein WM1.2 [<i>Aegilops tauschii</i>] (evaluate: 8.4e-15, score=82.8)	1.67	0.02
SGN-U227625	arabidopsis/peptide: At3g14460.1 68410.m01642 disease resistance protein (NBS-LRR class), putative domain signature NBS-LRR exists, suggestive of a disease resistance protein (evaluate: 9.6e-25, score=110.2) genbank/nr: gi 32470648 gb AAP45174.1 putative disease resistant protein rga4 [<i>Solanum bulbocastanum</i>] (evaluate: 2e-114, score=413.3)	1.45	0.09
X56040	<i>L.esculentum</i> TSW12 mRNA	2.13	0.03
DETOXIFICATION			
SGN-U219160	arabidopsis/peptide: At4g24750.1 68411.m03227 expressed protein (evaluate: 5e-72, score=268.5) genbank/nr: gi 46805304 dbj BAD16836.1 rhodanese-like domain-containing protein -like [<i>Oryza sativa</i> (japonica cultivar-group)] >gi 47847819 dbj BAD21614.1 rhodanese-like domain-containing protein -like [<i>Oryza sativa</i> (japonica cultivar-group)] (evaluate: 1.9e-78, score=295)	2.1	0
SGN-U220749	arabidopsis/peptide: At3g08920.1 68410.m00934 rhodanese-like domain protein contains rhodanese-like domain PF:00581 (evaluate: 4.5e-68, score=254.6) genbank/nr: gi 18491227 gb AAL69515.1 putative rhodanese family protein [<i>Arabidopsis thaliana</i>] (evaluate: 1.7e-66, score=254.6)	1.53	0.03
ENERGY PATHWAYS			
SGN-U213052	arabidopsis/peptide: At1g79750.1 68408.m08532 malate oxidoreductase -related similar to malate oxidoreductase (NADP-dependent malic enzyme) GB:P34105 (<i>Populus balsamifera</i> subsp. <i>trichocarpa</i>) (evaluate: 0, score=918.3) genbank/nr: gi 7431232 pir T06402 malate dehydrogenase (oxaloacetate-decarboxylating) (NADP) (EC 1.1.1.40) 2, cytosolic - tomato gi 2150029 gb AAB58728.1 cytosolic NADP-malic enzyme [<i>Lycopersicon esculentum</i>] (evaluate: 0, score=1133.2)	2.03	0.01
SGN-U213366	arabidopsis/peptide: At2g39770.1 68409.m04425 GDP-mannose pyrophosphorylase identical to GDP-mannose pyrophosphorylase from <i>Arabidopsis thaliana</i> [GI:3598958]; updated per Conklin PL et al, PNAS 1999, 96(7):4198-203 (evaluate: 0, score=654.8) genbank/nr: gi 47681456 gb AAT37498.1 GDP-mannose pyrophosphorylase [<i>Lycopersicon esculentum</i>] (evaluate: 0, score=718.4)	1.54	0.06

... continued ...

TABLE 1 Cont.

SGN-U214009	arabidopsis/peptide: At1g23800.1 68408.m02717 mitochondrial aldehyde dehydrogenase (ALDH3) similar to mitochondrial aldehyde dehydrogenase ALDH3[Arabidopsis thaliana] gi 19850249 gb AAL99612 (evaluate: 3e-102, score=367.9) genbank/nr: gi 20530131 dbj BAB92019.1 mitochondrial aldehyde dehydrogenase [Sorghum bicolor] (evaluate: 3e-105, score=383.3)	1.98	0.04
SGN-U214299	arabidopsis/peptide: At3g46170.1 68410.m04627 short-chain dehydrogenase/reductase family protein contains similarity to 3-oxoacyl-[acyl-carrier protein] reductase SP:P51831 from [Bacillus subtilis] (evaluate: 7e-46, score=126.3) genbank/nr: gi 15231362 ref NP_190203.1 short-chain dehydrogenase/reductase family protein [Arabidopsis thaliana] gi 11250534 pir T49258 dehydrogenase-like protein - Arabidopsis thaliana gi 7799005 emb CAB90944.1 dehydrogenase-like protein [Arabidopsis thaliana] (evaluate: 2.4e-44, score=137.1)	1.52	0.04
SGN-U215938	arabidopsis/peptide: At4g15530.1 68411.m02150 pyruvate,orthophosphate dikinase (evaluate: 0, score=867.5) genbank/nr: gi 3024426 sp Q42910 PODK_MESCR Pyruvate,phosphate dikinase, chloroplast precursor (Pyruvate,orthophosphate dikinase) gi 1084302 pir S55478 pyruvate, phosphate dikinase (EC 2.7.9.1) - common ice plant gi 854265 emb CAA57872.1 pyruvate,orthophosphate dikinase [Mesembryanthemum crystallinum] (evaluate: 0, score=902.5)	1.98	0.04
SGN-U216270	arabidopsis/peptide: At4g16820.1 68411.m02311 lipase (class 3) family similar to DEFECTIVE IN ANTHER DEHISCENCE1 [Arabidopsis thaliana] GI:16215706; contains Pfam profile PF01764: Lipase (evaluate: 1.6e-71, score=266.2) genbank/nr: gi 18414755 ref NP_567515.1 lipase (class 3) family [Arabidopsis thaliana] (evaluate: 6.1e-70, score=266.2)	1.54	0.07
SGN-U216555	arabidopsis/peptide: At1g51650.1 68408.m05341 epsilon subunit of mitochondrial F1-ATPase identical to epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] GI:1655486 (evaluate: 2.9e-28, score=121.7) genbank/nr: gi 15217996 ref NP_175576.1 epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] gi 2493052 sp Q96253 ATP5_ARATH ATP synthase epsilon chain, mitochondrial gi 25290492 pir C96555 protein epsilon subunit of mitochondrial F1-ATPase [imported] - Arabidopsis thaliana gi 1655486 dbj BAA13602.1 epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] gi 12321688 gb AAG50890.1 epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] gi 18252167 gb AAL61916.1 epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] gi 21386911 gb AAM47859.1 epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] (evaluate: 9.9e-27, score=121.7)	1.52	0

... continued ...

TABLE 1 Cont.

SGN-U226422	arabidopsis/peptide: At2g43130.1 68409.m04843 Ras-related GTP-binding protein (ARA-4) identical to SP:P28187 from [Arabidopsis thaliana] (evalue: 8.8e-20, score=92.82) genbank/nr: gi 7438428 pir T06443 GTP-binding protein - garden pea gi 303730 dbj BAA02108.1 GTP-binding protein [Pisum sativum] gi 738933 prf 2001457A GTP-binding protein (evalue: 1.1e-18, score=93.97)	1.84	0.07
HORMONE RESPONSE			
L16582	Lycopersicon esculentum arginine decarboxylase mRNA, complete cds	1.48	0.04
SGN-U212804	arabidopsis/peptide: At1g06620.1 68408.m00635 2-oxoglutarate-dependent dioxygenase, putative similar to 2A6 (GI:599622) and tomato ethylene synthesis regulatory protein E8 (SPI10967); contains Pfam profile: PF00671 Iron/Ascorbate oxidoreductase family (evalue: 1e-106, score=383.6) genbank/nr: gi 119640 sp P10967 ACC3_LYCES 1-aminocyclopropane-1-carboxylate oxidase homolog (Protein E8) gi 82109 pir S01642 ripening protein E8 - tomato gi 19199 emb CAA31789.1 E8 protein [Lycopersicon esculentum] (evalue: 0, score=718.8)	2.6	0.02
SGN-U214005	arabidopsis/peptide: At1g66340.1 68408.m06909 ethylene-response protein, ETR1 identical to GB:P49333 from [Arabidopsis thaliana] (Science 262 (5133), 539-544 (1993)) (evalue: 7.9e-11, score=64.31) genbank/nr: gi 7488991 pir T07026 ethylene receptor - tomato (strain Ailsa Craig) (fragment) gi 984157 emb CAA90808.1 ethylene receptor [Lycopersicon esculentum] (evalue: 1.3e-33, score=145.2)	1.53	0.07
SGN-U222167	arabidopsis/peptide: At2g46370.2 68409.m05709 auxin-responsive protein family similar to auxin-responsive GH3 product [Glycine max] GI:18591; contains Pfam profile PF03321: GH3 auxin-responsive promoter (evalue: 0, score=636.3) genbank/nr: gi 48843811 gb AAT47070.1 putative auxin-regulated protein [Oryza sativa (japonica cultivar-group)] (evalue: 0, score=636.3)	1.79	0.09
LIPID METABOLISM			
SGN-U213442	arabidopsis/peptide: At5g10170.1 68412.m01086 myo-inositol-1-phosphate synthase -related protein myo-inositol-1-phosphate synthase, Nicotiana paniculata, EMBL:AB032073 (evalue: 0, score=916) genbank/nr: gi 14548096 sp Q9LW96 INO1_TOBAC Inositol-3-phosphate synthase (Myo-inositol-1-phosphate synthase) (MI-1-P synthase) (IPS) gi 8096266 dbj BAA95788.1 myo-inositol 1-phosphate synthase [Nicotiana tabacum] (evalue: 0, score=974.5)	1.72	0.01

... continued ...

TABLE 1 Cont.

SGN-U214575	arabidopsis/peptide: At5g01650.1 68412.m00074 Macrophage migration inhibitory factor (MIF) family contains pfam profile: PF001187 Macrophage migration inhibitory factor (evaluate: 1.1e-54, score=209.5) genbank/nr: gi 15241023 ref NP_195785.1 Macrophage migration inhibitory factor (MIF) family [Arabidopsis thaliana] gi 11281345 pir T48186 light-inducible protein ATLS1 - Arabidopsis thaliana gi 7327824 emb CAB82281.1 light-inducible protein ATLS1 [Arabidopsis thaliana] gi 17065566 gb AAL32937.1 light-inducible protein ATLS1 [Arabidopsis thaliana] gi 20148493 gb AAM10137.1 light-inducible protein ATLS1 [Arabidopsis thaliana] (evaluate: 3.7e-53, score=209.5)	1.73	0.07
SGN-U216352	arabidopsis/peptide: At2g45790.1 68409.m05148 phosphomannomutase -related (evaluate: 4e-116, score=414.8) genbank/nr: gi 38345559 emb CAE03433.2 OSJNBa0032F06.16 [Oryza sativa (japonica cultivar-group)] (evaluate: 9e-119, score=428.7)	1.76	0.06
SGN-U217053	arabidopsis/peptide: At5g48370.1 68412.m05388 thioesterase family similar to SP Q9R0X4 48 kDa acyl-CoA thioester hydrolase, mitochondrial precursor (EC 3.1.2.-) {Mus musculus}; contains Pfam profile PF03061: thioesterase family protein (evaluate: 0, score=639.4) genbank/nr: gi 15238956 ref NP_199648.1 thioesterase family [Arabidopsis thaliana] gi 8978341 dbj BAA98194.1 contains similarity to acyl-CoA thioesterase~gene_id:K23F3.9 [Arabidopsis thaliana] (evaluate: 0, score=639.4)	1.56	0.03
METABOLISM			
SGN-U212562	arabidopsis/peptide: At5g17330.1 68412.m01869 glutamate decarboxylase 1 (GAD 1) sp Q42521 (evaluate: 1e-140, score=495.7) genbank/nr: gi 7436483 pir T01962 glutamate decarboxylase (EC 4.1.1.15) 2, calmodulin-binding - common tobacco gi 3252854 gb AAC39483.1 glutamate decarboxylase isozyme 2 [Nicotiana tabacum] (evaluate: 3e-144, score=512.7)	1.79	0.05
SGN-U214499	arabidopsis/peptide: At4g32940.1 68411.m04261 gamma-VPE (vacuolar processing enzyme) (evaluate: 0, score=714.5) genbank/nr: gi 27544012 dbj BAC54830.1 vacuolar processing enzyme-3 [Nicotiana tabacum] (evaluate: 0, score=866.7)	1.65	0.02
SGN-U216737	arabidopsis/peptide: At1g03150.1 68408.m00261 GCN5-related N-acetyltransferase (GNAT) family similar to SP P07347 N-terminal acetyltransferase complex ARD1 subunit (Arrest-defective protein 1) {Saccharomyces cerevisiae}; contains Pfam profile PF00583: acetyltransferase, GNAT family (evaluate: 1.3e-84, score=309.7) genbank/nr: gi 18379062 ref NP_563677.1 GCN5-related N-acetyltransferase (GNAT) family [Arabidopsis thaliana] gi 21536510 gb AAM60842.1 unknown [Arabidopsis thaliana] (evaluate: 4.6e-83, score=309.7)	2.01	0.1

... continued ...

TABLE 1 Cont.

SGN-U218165	arabidopsis/peptide: At1g32900.1 68408.m03672 starch synthase, putative similar to starch synthase SP:Q42857 from [<i>Ipomoea batatas</i>] (evalue: 2e-130, score=462.6) genbank/nr: gi 267196 sp Q00775 SSG1_SOLTU Granule-bound starch synthase I, chloroplast precursor (GBSS I) >gi 66574 pir YUPOY starch synthase (EC 2.4.1.21) precursor - potato >gi 21471 emb CAA41359.1 glycogen (starch) synthase [<i>Solanum tuberosum</i>] (evalue: 2e-167, score=590.9)	1.74	0.04
OTHERS			
AF320028	<i>Lycopersicon esculentum</i> heme oxygenase 1 (HO1) gene, complete cds; nuclear gene for chloroplast product	1.64	0.02
SGN-U213852	arabidopsis/peptide: At3g48740.1 68410.m04926 nodulin MtN3 family protein similar to MtN3 GI:1619602 (root nodule development) from [<i>Medicago truncatula</i>] (evalue: 3.4e-77, score=285) genbank/nr: gi 15229019 ref NP_190443.1 nodulin MtN3 family protein [<i>Arabidopsis thaliana</i>] gi 11282600 pir T46218 MTN3-like protein - <i>Arabidopsis thaliana</i> gi 6523105 emb CAB62363.1 MTN3-like protein [<i>Arabidopsis thaliana</i>] gi 13605688 gb AAK32837.1 AT3g48740/T8P19_250 [<i>Arabidopsis thaliana</i>] gi 16930411 gb AAL31891.1 AT3g48740/T8P19_250 [<i>Arabidopsis thaliana</i>] gi 17979365 gb AAL49908.1 putative MTN3 protein [<i>Arabidopsis thaliana</i>] gi 18700264 gb AAL77742.1 AT3g48740/T8P19_250 [<i>Arabidopsis thaliana</i>] gi 20465523 gb AAM20244.1 putative MTN3 protein [<i>Arabidopsis thaliana</i>] (evalue: 1.3e-75, score=285)	2.05	0.04
SGN-U213959	arabidopsis/peptide: At1g04580.1 68408.m00408 aldehyde oxidase, putative similar to aldehyde oxidases from <i>Arabidopsis thaliana</i> : GI:3172023, GI:3172025, GI:3172044 (evalue: 3e-89, score=325.1) genbank/nr: gi 10764218 gb AAG22607.1 aldehyde oxidase [<i>Lycopersicon esculentum</i>] gi 14028575 gb AAK52410.1 aldehyde oxidase TAO3 [<i>Lycopersicon esculentum</i>] (evalue: 1e-138, score=494.2)	1.45	0.06
SGN-U214814	arabidopsis/peptide: At2g06050.2 68415.m00664 12-oxophytodienoate reductase (OPR3) / delayed dehiscence1 (DDE1) nearly identical to DELAYED DEHISCENCE1 [GI:7688991] and to OPR3 [GI:10242314]; contains Pfam profile PF00724:oxidoreductase, FAD/FMN-binding; identical to cDNA OPDA-reductase homolog GI:5059114 (evalue: 3e-172, score=602.1) genbank/nr: gi 12056507 emb CAC21424.1 12-oxophytodienoate reductase 3 [<i>Lycopersicon esculentum</i>] (evalue: 0, score=801.2)	1.52	0.07

... continued ...

TABLE 1 Cont.

SGN-U215281	arabidopsis/peptide: At2g36690.1 68409.m04074 oxidoreductase, 2OG-Fe(II) oxygenase family similar to IDS3 [Hordeum vulgare][GI:4514655], leucoanthocyanidin dioxygenase [SP P51091][Malus domestica]; contains PF03171 2OG-Fe(II) oxygenase superfamily domain (evaluate: 9e-63, score=237.7) genbank/nr: gi 42408583 dbj BAD09760.1 putative flavonol synthase [Oryza sativa (japonica cultivar-group)] >gi 42409017 dbj BAD10270.1 putative flavonol synthase [Oryza sativa (japonica cultivar-group)] (evaluate: 9.3e-67, score=256.1)	1.53	0.06
SGN-U215649	arabidopsis/peptide: At2g03760.1 68409.m00299 steroid sulfotransferase, putative strong similarity to steroid sulfotransferases from [Brassica napus] GI:3420008, GI:3420004, GI:3420006; contains Pfam profile PF00685: Sulfotransferase domain (evaluate: 3.3e-92, score=335.5) genbank/nr: gi 15227699 ref NP_178471.1 steroid sulfotransferase, putative [Arabidopsis thaliana] gi 27735199 sp P52839 FSTL_ARATH Flavonol sulfotransferase-like (RaRO47) gi 25288807 pir A84452 probable steroid sulfotransferase [imported] - Arabidopsis thaliana gi 4406767 gb AAD20078.1 putative steroid sulfotransferase [Arabidopsis thaliana] gi 14030735 gb AAK53042.1 At2g03760/F19B11.21 [Arabidopsis thaliana] gi 21360485 gb AAM47358.1 At2g03760/F19B11.21 [Arabidopsis thaliana] (evaluate: 1.3e-90, score=335.5)	2.95	0.08
SGN-U215928	arabidopsis/peptide: At4g22220.1 68411.m02939 iron-sulfur cluster assembly complex protein, putative (ISCU1) similar to iron-sulfur cluster assembly complex ISCU1 (GI:11545705) [Homo sapiens]; nifU protein homolog YPL135w (GI:15619823) [Saccharomyces cerevisiae] PIR2:S69049 (evaluate: 3.2e-65, score=245) genbank/nr: gi 34912076 ref NP_917385.1 putative nifU-like protein [Oryza sativa (japonica cultivar-group)] gi 20521223 dbj BAB91740.1 putative nifU-like protein [Oryza sativa (japonica cultivar-group)] (evaluate: 1.6e-65, score=251.1)	1.74	0.07
SGN-U216025	arabidopsis/peptide: At5g19300.1 68412.m02105 hypothetical protein predicted proteins, H. sapiens, D. melanogaster and others (evaluate: 2e-120, score=429.5) genbank/nr: gi 42567956 ref NP_197431.2 expressed protein [Arabidopsis thaliana] >gi 45825145 gb AAS77480.1 At5g19300 [Arabidopsis thaliana] (evaluate: 8e-119, score=429.5)	1.7	0.01
SGN-U216398	arabidopsis/peptide: At4g28730.1 68411.m03744 glutaredoxin protein family contains glutaredoxin domain, Pfam:PF00462 (evaluate: 2.9e-46, score=182.2) genbank/nr: gi 30688093 ref NP_194602.2 glutaredoxin protein family [Arabidopsis thaliana] gi 26452363 dbj BAC43267.1 unknown protein [Arabidopsis thaliana] gi 28372898 gb AAO39931.1 At4g28730 [Arabidopsis thaliana] (evaluate: 1.1e-44, score=182.2)	1.73	0.02

... continued ...

TABLE 1 Cont.

SGN-U217201	arabidopsis/peptide: At4g25630.1 68411.m03360 fibrillarlin 2 (AtFib2) identical to fibrillarlin 2 GI:9965655 from [Arabidopsis thaliana] (evalue: 2e-124, score=441.8) genbank/nr: gi 18416588 ref NP_567724.1 fibrillarlin 2 (AtFib2) [Arabidopsis thaliana] gi 7450791 pir T09555 fibrillarlin - Arabidopsis thaliana gi 4914455 emb CAB43694.1 fibrillarlin-like protein [Arabidopsis thaliana] gi 7269413 emb CAB81373.1 fibrillarlin-like protein [Arabidopsis thaliana] gi 9965655 gb AAG10104.1 fibrillarlin 2 [Arabidopsis thaliana] gi 9965796 gb AAG10153.1 fibrillarlin 2 [Arabidopsis thaliana] gi 21536840 gb AAM61172.1 fibrillarlin 2 (AtFib2) [Arabidopsis thaliana] gi 23297150 gb AAN13105.1 fibrillarlin 2 (AtFib2) [Arabidopsis thaliana] (evalue: 7e-123, score=441.8)	1.71	0.1
SGN-U217291	arabidopsis/peptide: At5g14520.1 68412.m01569 pescadillo - like protein embryonic development allele: hi2, Danio rerio, EMBL:U77627pescadillo, Homo sapiens, EMBL:U78310 (evalue: 3e-160, score=562) genbank/nr: gi 28273368 gb AAO38454.1 pescadillo-like protein [Oryza sativa (japonica cultivar-group)] (evalue: 8e-162, score=572.4)	1.53	0.08
SGN-U218491	arabidopsis/peptide: At2g31740.1 68409.m03514 expressed protein (evalue: 1.9e-61, score=233.4) genbank/nr: gi 34148076 gb AAQ62585.1 putative spermine/spermidine synthase [Glycine max] (evalue: 2.6e-73, score=278.1)	1.59	0.07
SGN-U219582	arabidopsis/peptide: At4g37470.1 68411.m04822 hydrolase, alpha/beta fold family low similarity to SP Q59093 3-oxoadipate enol-lactonase I (EC 3.1.1.24) (Enol-lactone hydrolase I) (Beta-ketoadipate enol-lactone hydrolase I) {Acinetobacter calcoaceticus}; contains Pfam profile PF00561: hydrolase, alpha/beta fold family (evalue: 2e-124, score=442.2) genbank/nr: gi 15235567 ref NP_195463.1 hydrolase, alpha/beta fold family [Arabidopsis thaliana] gi 7450663 pir T04741 hypothetical protein F6G17.120 - Arabidopsis thaliana gi 4468813 emb CAB38214.1 putative protein [Arabidopsis thaliana] gi 7270729 emb CAB80412.1 putative protein [Arabidopsis thaliana] gi 15810303 gb AAL07039.1 unknown protein [Arabidopsis thaliana] gi 20259141 gb AAM14286.1 unknown protein [Arabidopsis thaliana] (evalue: 8e-123, score=442.2)	1.57	0.06
SGN-U221073	arabidopsis/peptide: At5g42970.1 68412.m04727 COP9 complex subunit, FUS4 FUSCA4, COP8, CSN4; identical to COP8 GI:5802627 from [Arabidopsis thaliana] (evalue: 1.7e-74, score=275.4) genbank/nr: gi 33324486 gb AAQ07984.1 COP8-like protein [Lilium longiflorum] (evalue: 2.4e-74, score=280)	1.64	0.06
SGN-U221084	arabidopsis/peptide: At1g60680.1 68408.m06265 aldo/keto reductase family contains Pfam profile PF00248: oxidoreductase, aldo/keto reductase family (evalue: 1.4e-51, score=199.5) genbank/nr: gi 37534402 ref NP_921503.1 putative polyprotein [Oryza sativa (japonica cultivar-group)] gi 31432120 gb AAP53790.1 putative polyprotein [Oryza sativa (japonica cultivar-group)] (evalue: 1.5e-62, score=241.1)	2.05	0.09

... continued ...

TABLE 1 Cont.

SGN-U221863	arabidopsis/peptide: At3g50210.1 68410.m05087 oxidoreductase (din11), putative strong similarity to partial cds of 2-oxoacid-dependent oxidase (din11) from GI:10834554 [Arabidopsis thaliana] (evalue: 1.2e-63, score=239.2) genbank/nr: gi 6984228 gb AAF34802.1 putative flavonol synthase-like protein [Euphorbia esula] (evalue: 8.8e-68, score=258.1)	1.72	0.06
SGN-U221959	arabidopsis/peptide: At2g47880.1 68409.m05397 glutaredoxin protein family contains INTERPRO Domain IPR002109, Glutaredoxin (thioltransferase) (evalue: 2.9e-36, score=149.4) genbank/nr: gi 15227151 ref NP_182309.1 glutaredoxin protein family [Arabidopsis thaliana] gi 25282784 pir F84920 probable glutaredoxin [imported] - Arabidopsis thaliana gi 3738300 gb AAC63642.1 putative glutaredoxin [Arabidopsis thaliana] gi 20197557 gb AAM15127.1 putative glutaredoxin [Arabidopsis thaliana] gi 21554200 gb AAM63279.1 putative glutaredoxin [Arabidopsis thaliana] (evalue: 1.1e-34, score=149.4)	2.11	0.06
SGN-U223643	arabidopsis/peptide: At2g42490.1 68409.m04750 copper amine oxidase -related (evalue: 2e-170, score=594.7) genbank/nr: gi 5230728 gb AAD40979.1 peroxisomal copper-containing amine oxidase [Glycine max] (evalue: 7e-175, score=615.1)	2.02	0.05
SGN-U223935	arabidopsis/peptide: At4g08790.1 68411.m01305 nitrilase 1 like protein nitrilase 1 - Mus musculus,PID:g3228668 (evalue: 9e-123, score=436.8) genbank/nr: gi 18413157 ref NP_567340.1 nitrilase 1 like protein [Arabidopsis thaliana] gi 13926307 gb AAK49620.1 AT4g08790/T32A17_100 [Arabidopsis thaliana] gi 22137058 gb AAM91374.1 At4g08790/T32A17_100 [Arabidopsis thaliana] (evalue: 3e-121, score=436.8)	1.54	0.06
SGN-U225236	arabidopsis/peptide: At3g26040.1 68410.m02970 acyltransferase family similar to deacetylvindoline 4-O-acetyltransferase [Catharanthus roseus][GI:4091808][PMID:9681034], alcohol acyltransferase [Fragaria x ananassa][GI:10121328][PMID:10810141] (evalue: 1.2e-29, score=127.1) genbank/nr: gi 48210045 gb AAT40544.1 putative acetyltransferase [Solanum demissum] (evalue: 3e-29, score=131)	1.7	0.09
OXIDATIVE BURST / HYPERSENSITIVE RESPONSE			
SGN-U212914	arabidopsis/peptide: At1g08830.1 68408.m00884 copper/zinc superoxidase dismutase (CSD1) identical to SWISS-PROT: P24704 (evalue: 4.6e-70, score=261.2) genbank/nr: gi 3334337 sp Q43779 SOD2_LYCES Superoxide dismutase [Cu-Zn] 2 gi 1084402 pir S55402 superoxide dismutase (EC 1.15.1.1) (Cu-Zn), cytosolic - tomato gi 854248 emb CAA60826.1 cytosolic Cu,Zn superoxide dismutase [Lycopersicon esculentum] (evalue: 1.2e-79, score=298.1)	1.63	0.1
SGN-U213351	arabidopsis/peptide: At1g71695.1 68408.m07598 peroxidase, putative identical to GB:CAA67309 GI:1429213 from [Arabidopsis thaliana] (evalue: 1.1e-83, score=307) genbank/nr: gi 14031049 gb AAK52084.1 peroxidase [Nicotiana tabacum] (evalue: 4.7e-91, score=336.7)	2.11	0.11

... continued ...

TABLE 1 Cont.

SGN-U214733	arabidopsis/peptide: At4g11600.1 68411.m01682 glutathione peroxidase, putative (evalue: 8.2e-79, score=290.4) genbank/nr: gi 20138152 sp O24031 GPX4_LYCES Probable phospholipid hydroperoxide glutathione peroxidase (PHGPx) gi 2388885 emb CAA75054.1 glutathione peroxidase [<i>Lycopersicon esculentum</i>] (evalue: 1.8e-93, score=344.4)	1.61	0.06
SGN-U214812	arabidopsis/peptide: At4g31870.1 68411.m04123 glutathione peroxidase, putative glutathione peroxidase, <i>Arabidopsis thaliana</i> , PIR2:S71250 (evalue: 5.9e-86, score=314.3) genbank/nr: gi 20138099 sp O24296 GPX1_PEA Phospholipid hydroperoxide glutathione peroxidase, chloroplast precursor (PHGPx) gi 7433115 pir T06462 glutathione peroxidase (EC 1.11.1.9) precursor - garden pea gi 2632109 emb CAA04142.1 phospholipid glutathione peroxidase [<i>Pisum sativum</i>] (evalue: 7.6e-85, score=315.8)	1.41	0.07
SGN-U216820	arabidopsis/peptide: At1g63460.1 68408.m06583 glutathione peroxidase, putative contains Pfam profile: PF00255 glutathione peroxidases (evalue: 4.7e-71, score=264.2) genbank/nr: gi 18407822 ref NP_564813.1 glutathione peroxidase, putative [<i>Arabidopsis thaliana</i>] gi 21592603 gb AAM64552.1 unknown [<i>Arabidopsis thaliana</i>] gi 27765006 gb AAO23624.1 At1g63460 [<i>Arabidopsis thaliana</i>] (evalue: 1.7e-69, score=264.2)	1.52	0.31
SGN-U219084	arabidopsis/peptide: At2g48150.1 68409.m05437 glutathione peroxidase, putative (evalue: 7e-69, score=257.3) genbank/nr: gi 18407538 ref NP_566128.1 glutathione peroxidase, putative [<i>Arabidopsis thaliana</i>] gi 21617962 gb AAM67012.1 putative glutathione peroxidase [<i>Arabidopsis thaliana</i>] gi 26451929 dbj BAC43057.1 putative glutathione peroxidase [<i>Arabidopsis thaliana</i>] gi 28372962 gb AAO39963.1 At2g48150 [<i>Arabidopsis thaliana</i>] (evalue: 2.6e-67, score=257.3)	1.6	0.01
SGN-U219399	arabidopsis/peptide: At1g44970.1 68408.m04722 peroxidase, putative similar to peroxidase GI:993004 from [<i>Mercurialis annua</i>] (evalue: 3e-137, score=485) genbank/nr: gi 14031051 gb AAK52085.1 peroxidase [<i>Nicotiana tabacum</i>] (evalue: 9e-155, score=548.5)	1.62	0.06
SGN-U212749	arabidopsis/peptide: At3g09270.1 68410.m00987 glutathione transferase, putative similar to glutathione transferase GB:CAA71784 [<i>Glycine max</i>] (evalue: 4.6e-51, score=197.6) genbank/nr: gi 416649 sp Q03662 GTX1_TOBAC Probable glutathione S-transferase (Auxin-induced protein PGNT1/PCNT110) gi 100303 pir S16267 auxin-induced protein (clones pGNT1 and pCNT110) - common tobacco gi 19789 emb CAA39709.1 auxin-induced protein [<i>Nicotiana tabacum</i>] gi 19795 emb CAA39705.1 auxin-induced protein [<i>Nicotiana tabacum</i>] (evalue: 1.1e-82, score=307.8)	2.6	0.03
SGN-U215385	arabidopsis/peptide: At1g71695.1 68408.m07598 peroxidase, putative identical to GB:CAA67309 GI:1429213 from [<i>Arabidopsis thaliana</i>] (evalue: 3e-116, score=415.2) genbank/nr: gi 14031049 gb AAK52084.1 peroxidase [<i>Nicotiana tabacum</i>] (evalue:	1.67	0.01

... continued ...

TABLE 1 Cont.

	3e-166, score=587)		
SGN-U215794	arabidopsis/peptide: At2g28190.1 68409.m03098 copper/zinc superoxide dismutase (CSD2) identical to GP:3273753:AF061519 (evalue: 4.3e-67, score=251.5) genbank/nr: gi 33327349 gb AAQ09007.1 superoxidase dismutase [<i>Lycopersicon esculentum</i>] (evalue: 2.7e-81, score=303.9)	1.44	0.1
SGN-U216874	arabidopsis/peptide: At1g06130.2 68408.m08983 glyoxalase II, putative (hydroxyacylglutathione hydrolase) similar to glyoxalase II isozyme GB:AAC49865 GI:2570338 from [<i>Arabidopsis thaliana</i>] (evalue: 1e-124, score=443.7) genbank/nr: gi 30679573 ref NP_849599.1 glyoxalase II, putative (hydroxyacylglutathione hydrolase) [<i>Arabidopsis thaliana</i>] gi 20466237 gb AAM20436.1 glyoxalase II isozyme, putative [<i>Arabidopsis thaliana</i>] gi 22136310 gb AAM91233.1 glyoxalase II isozyme, putative [<i>Arabidopsis thaliana</i>] (evalue: 4e-123, score=443.7)	1.62	0.05
SGN-U216884	arabidopsis/peptide: At5g41210.1 68412.m04513 glutathione transferase, putative similar to emb CAA10662 (evalue: 1.3e-92, score=336.7) genbank/nr: gi 11385461 gb AAG34813.1 glutathione S-transferase GST 23 [<i>Glycine max</i>] (evalue: 5.2e-96, score=353.2)	2.18	0.01
SGN-U225169	arabidopsis/peptide: At5g04660.1 68412.m00439 cytochrome P450, putative cytochrome P450 77A3p, <i>Glycine max.</i> , PIR:T05948 (evalue: 1e-62, score=236.1) genbank/nr: gi 584867 sp P37124 C772_SOLME Cytochrome P450 77A2 (CYPLXXVIA2) (P-450EG5) gi 542071 pir S41598 cytochrome P450 77A2 - eggplant gi 438241 emb CAA50646.1 CYP77A2 [<i>Solanum melongena</i>] (evalue: 3.3e-83, score=309.3)	1.91	0.04
PHOTOSYNTHESIS			
SGN-U215807	arabidopsis/peptide: At1g01300.1 68408.m00037 chloroplast nucleoid DNA binding protein -related similar to chloroplast nucleoid DNA binding protein GB:BAA22813 GI:2541876 from [<i>Nicotiana tabacum</i>] (evalue: 2e-158, score=556.2) genbank/nr: gi 15223368 ref NP_171637.1 chloroplast nucleoid DNA binding protein -related [<i>Arabidopsis thaliana</i>] gi 25518405 pir C86143 hypothetical protein F6F3.10 - <i>Arabidopsis thaliana</i> gi 9665144 gb AAF97328.1 Unknown protein [<i>Arabidopsis thaliana</i>] gi 22135930 gb AAM91547.1 chloroplast nucleoid DNA binding protein, putative [<i>Arabidopsis thaliana</i>] gi 30387595 gb AAP31963.1 At1g01300 [<i>Arabidopsis thaliana</i>] (evalue: 6e-157, score=556.2)	1.59	0
SGN-U215851	arabidopsis/peptide: At3g15360.1 68410.m01749 thioredoxin M-type 4, chloroplast precursor (TRX-M4) nearly identical to SP Q9SEU6 Thioredoxin M-type 4, chloroplast precursor (TRX-M4) [<i>Arabidopsis thaliana</i>] (evalue: 2.2e-46, score=182.6) genbank/nr: gi 15594012 emb CAC69854.1 putative thioredoxin m2 [<i>Pisum sativum</i>] (evalue: 1.9e-54, score=214.5)	1.77	0.05

... continued ...

TABLE 1 Cont.

SGN-U218193	arabidopsis/peptide: At5g27380.1 68412.m02976 glutathione synthetase (GSH2) non-consensus AT donor splice site at exon 6, AC acceptor splice site at exon 7 (evalue: 0, score=686.8) genbank/nr: gi 20138145 sp O22494 GSHB_LYCES Glutathione synthetase, chloroplast precursor (Glutathione synthase) (GSH synthetase) (GSH-S) gi 7489006 pir T04336 glutathione synthase (EC 6.3.2.3) 2 - tomato gi 2407617 gb AAB71231.1 glutathione synthetase [<i>Lycopersicon esculentum</i>] (evalue: 0, score=1070.5)	1.48	0.1
PROTEIN BIOSYNTHESIS			
SGN-U212746	arabidopsis/peptide: At2g16600.1 68409.m01716 cytosolic cyclophilin (ROC3) (evalue: 6.6e-74, score=273.9) genbank/nr: gi 118103 sp P21568 CYPH_LYCES Peptidyl-prolyl cis-trans isomerase (PPIase) (Rotamase) (Cyclophilin) (Cyclosporin A-binding protein) gi 170440 gb AAA63543.1 cyclophilin (evalue: 1.4e-88, score=327.8)	1.59	0.03
SGN-U212967	arabidopsis/peptide: At2g27530.2 68409.m05661 60S ribosomal protein L10A (RPL10aB) (evalue: 7.9e-97, score=350.5) genbank/nr: gi 18401451 ref NP_565654.1 60S ribosomal protein L10A (RPL10aB) [<i>Arabidopsis thaliana</i>] gi 30683566 ref NP_850104.1 60S ribosomal protein L10A (RPL10aB) [<i>Arabidopsis thaliana</i>] gi 27923989 sp P59230 R10B_ARATH 60S ribosomal protein L10a-2 gi 13430468 gb AAK25856.1 putative 60S ribosomal protein L10A [<i>Arabidopsis thaliana</i>] gi 15810665 gb AAL07257.1 putative 60S ribosomal protein L10A [<i>Arabidopsis thaliana</i>] gi 19698833 gb AAL91152.1 60S ribosomal protein L10A [<i>Arabidopsis thaliana</i>] gi 20197452 gb AAC73045.2 60S ribosomal protein L10A [<i>Arabidopsis thaliana</i>] gi 20197665 gb AAM15190.1 60S ribosomal protein L10A [<i>Arabidopsis thaliana</i>] gi 30023674 gb AAP13370.1 At2g27530 [<i>Arabidopsis thaliana</i>] (evalue: 3e-95, score=350.5)	1.56	0.05
SGN-U213207	arabidopsis/peptide: At3g09200.1 68410.m00980 60S acidic ribosomal protein P0 (RPP0B) similar to putative 60S acidic ribosomal protein P0 GB:P50346 [Glycine max] (evalue: 1e-124, score=443.4) genbank/nr: gi 1710587 sp P50346 RLA0_SOYBN 60S ACIDIC RIBOSOMAL PROTEIN P0 gi 7440740 pir T07106 acidic ribosomal protein P0 - soybean gi 1196897 gb AAB63814.1 acidic ribosomal protein P0 [Glycine max] (evalue: 1e-125, score=451.8)	1.76	0.1
SGN-U213445	arabidopsis/peptide: At3g11510.1 68410.m01267 40S ribosomal protein S14 (RPS14B) similar to 40S ribosomal protein S14 GB:P19950 [<i>Zea mays</i>] (evalue: 8.4e-60, score=226.9) genbank/nr: gi 28436071 gb AAO41731.1 cytoplasmic ribosomal protein S14 [<i>Brassica napus</i>] (evalue: 1.2e-59, score=231.5)	1.41	0.01

... continued ...

TABLE 1 Cont.

SGN-U213502	arabidopsis/peptide: At3g13920.1 68410.m01577 eukaryotic translation initiation factor 4A-1 (eIF4A-1) eIF-4A-1 gi:15293046, gi:15450485 (evalue: 0, score=771.2) genbank/nr: gi 1170506 sp P41379 IF42_NICPL Eukaryotic initiation factor 4A-2 (eIF4A-2) (eIF-4A-2) gi 100275 pir S22578 translation initiation factor eIF-4A - curled-leaved tobacco gi 19697 emb CAA43513.1 nicotiana eukaryotic translation initiation factor 4A [Nicotiana plumbaginifolia] (evalue: 0, score=811.6)	1.82	0
SGN-U215228	arabidopsis/peptide: At4g16520.2 68411.m05465 symbiosis-related like protein (evalue: 6.5e-57, score=217.2) genbank/nr: gi 21615419 emb CAD33929.1 microtubule associated protein [Cicer arietinum] (evalue: 1.4e-57, score=224.6)	1.43	0.02
SGN-U215438	arabidopsis/peptide: At5g58710.1 68412.m06653 cyclophilin ROC7 (evalue: 1.7e-87, score=319.3) genbank/nr: gi 15237739 ref NP_200679.1 cyclophilin ROC7 [Arabidopsis thaliana] gi 11270328 pir T50838 peptidylprolyl isomerase (EC 5.2.1.8) ROC7 [similarity] - Arabidopsis thaliana gi 6180043 gb AAF05760.1 cyclophilin [Arabidopsis thaliana] gi 8843791 dbj BAA97339.1 cyclophilin [Arabidopsis thaliana] gi 15081670 gb AAK82490.1 AT5g58710/mzn1_160 [Arabidopsis thaliana] gi 20334834 gb AAM16173.1 AT5g58710/mzn1_160 [Arabidopsis thaliana] gi 21554366 gb AAM63473.1 cyclophilin ROC7 [Arabidopsis thaliana] (evalue: 6.3e-86, score=319.3)	1.61	0.09
SGN-U217073	arabidopsis/peptide: At3g06680.1 68410.m00718 60S ribosomal protein L29 (RPL29B) similar to 60S ribosomal protein L29 GB:P25886 from (Rattus norvegicus) (evalue: 1.3e-23, score=106.3) genbank/nr: gi 12231298 gb AAG49033.1 ripening regulated protein DDTFR19 [Lycopersicon esculentum] (evalue: 9.1e-25, score=115.2)	1.62	0.05
SGN-U219046	arabidopsis/peptide: At3g57080.1 68410.m05871 eukaryotic rpb5 RNA polymerase subunit family similar to SP P19388 DNA-directed RNA polymerase II 23 kDa polypeptide (EC 2.7.7.6) {Homo sapiens}; contains Pfam profiles PF03871: RNA polymerase Rpb5 N-terminal domain, PF01191: RNA polymerase Rpb5 C-terminal domain (evalue: 1.9e-65, score=246.1) genbank/nr: gi 15230206 ref NP_191267.1 eukaryotic rpb5 RNA polymerase subunit family [Arabidopsis thaliana] gi 11261045 pir T47768 hypothetical protein F24I3.160 - Arabidopsis thaliana gi 6911878 emb CAB72178.1 putative protein [Arabidopsis thaliana] gi 26452919 dbj BAC43537.1 unknown protein [Arabidopsis thaliana] gi 28973011 gb AAO63830.1 unknown protein [Arabidopsis thaliana] (evalue: 7.1e-64, score=246.1)	1.7	0.03
SGN-U223212	arabidopsis/peptide: At3g07880.1 68410.m00870 RHO GDP-dissociation inhibitor 1 -related similar to RHO GDP-dissociation inhibitor 1 GB:P19803 [Bos taurus] (evalue: 1.3e-47, score=187.2) genbank/nr: gi 7228160 emb CAB77025.1 putative Rho GDP dissociation inhibitor [Nicotiana tabacum] (evalue: 1.5e-95, score=351.7)	1.56	0.01

... continued ...

TABLE 1 Cont.

SGN-U225241	arabidopsis/peptide: At5g05920.1 68412.m00608 deoxyhypusine synthase (evalue: 1e-138, score=489.6) genbank/nr: gi 38503163 sp Q9AXR0 DHYS_LYCES Deoxyhypusine synthase >gi 12407775 gb AAG53641.1 deoxyhypusine synthase [Lycopersicon esculentum] (evalue: 3e-173, score=610.1)	1.71	0.03
PROTEIN DEGRADATION			
SGN-U212891	arabidopsis/peptide: At2g02760.1 68409.m00192 ubiquitin-conjugating enzyme 2 (UBC2) E2; identical to gi:2689242, SP:P42745 (evalue: 1.8e-86, score=315.8) genbank/nr: gi 8118527 gb AAF73016.1 ubiquitin conjugating protein [Avicennia marina] (evalue: 1.4e-85, score=318.2)	1.53	0.1
SGN-U213149	arabidopsis/peptide: At5g53300.2 68412.m07968 ubiquitin-conjugating enzyme 10 (UBC10) E2; identical to gi:297877, SP:P35133 (evalue: 1.3e-83, score=306.2) genbank/nr: gi 5762457 gb AAD51109.1 ubiquitin-conjugating enzyme UBC2 [Mesembryanthemum crystallinum] (evalue: 9.7e-83, score=308.5)	1.64	0.03
SGN-U213613	arabidopsis/peptide: At5g43580.1 68412.m04803 hypothetical protein (evalue: 3.6e-08, score=54.3) genbank/nr: gi 124192 sp P20076 IER1_LYCES Ethylene-responsive proteinase inhibitor 1 precursor gi 82085 pir A32067 ethylene-responsive proproteinase inhibitor 1 precursor - tomato gi 623594 gb AAA60745.1 proteinase inhibitor 1 (evalue: 1e-09, score=64.31)	2.36	0.04
SGN-U214642	arabidopsis/peptide: At1g45000.1 68408.m04725 26S proteasome regulatory particle triple-A ATPase subunit4 -related similar to 26S proteasome regulatory particle triple-A ATPase subunit4 GI:11094192 from [Oryza sativa] (evalue: 0, score=736.1) genbank/nr: gi 24745880 dbj BAC23035.1 26S proteasome AAA-ATPase subunit RPT4a [Solanum tuberosum] (evalue: 0, score=777.3)	2.09	0.01
SGN-U217107	arabidopsis/peptide: At3g45010.1 68410.m04491 serine carboxypeptidase III, putative similar to serine carboxypeptidase III from Oryza sativa SP P37891, Matricaria chamomilla GI:6960455, Hordeum vulgare SP P21529, Triticum aestivum SP P11515; contains Pfam profile PF0450 serine carboxypeptidase (evalue: 1.4e-99, score=359.4) genbank/nr: gi 6960455 gb AAD42963.2 serine carboxypeptidase precursor [Matricaria chamomilla] (evalue: 5e-101, score=369.4)	1.73	0.09
SGN-U219136	arabidopsis/peptide: At4g24690.1 68411.m03221 ubiquitin-associated (UBA)/PB1 domain-containing protein contains Pfam profiles PF00627: Ubiquitin-associated (UBA)/TS-N domain, PF00569: Zinc finger ZZ type domain, PF00564: PB1 domain (evalue: 2.6e-80, score=295.8) genbank/nr: gi 25044803 gb AAM28274.1 PFE18 protein [Ananas comosus] (evalue: 7.6e-79, score=296.2)	1.55	0.03
SGN-U219551	arabidopsis/peptide: At5g42220.1 68412.m04634 ubiquitin family contains INTERPRO:IPR000626 ubiquitin domain (evalue: 1e-70, score=264.6) genbank/nr: gi 10177007 dbj BAB10195.1 gene_id:K5J14.2~pir T30561~similar to unknown protein [Arabidopsis thaliana] (evalue: 3.9e-69, score=264.6)	1.71	0.09

... continued ...

TABLE 1 Cont.

SGN-U220585	arabidopsis/peptide: At1g22050.1 68408.m02495 ubiquitin family contains INTERPRO:IPR000626 ubiquitin domain (evaluate: 1.2e-37, score=153.7) genbank/nr: gi 4097587 gb AAD00119.1 NTGP5 [Nicotiana tabacum] (evaluate: 4.5e-49, score=196.8)	2.03	0.08
SGN-U220965	arabidopsis/peptide: At3g07990.1 68410.m00881 serine carboxypeptidase -related similar to serine carboxypeptidase II (CP-MII) GB:CAA70815 [Hordeum vulgare] (evaluate: 5.5e-82, score=300.8) genbank/nr: gi 15231911 ref NP_187456.1 serine carboxypeptidase -related [Arabidopsis thaliana] gi 6648211 gb AAF21209.1 putative serine carboxypeptidase II [Arabidopsis thaliana] (evaluate: 2e-80, score=300.8)	1.65	0.03
SIGNAL TRANSDUCTION			
SGN-U212665	arabidopsis/peptide: At3g51850.1 68410.m05270 calcium-dependent protein kinase, putative (CDPK) similar to calcium-dependent protein kinase [Arabidopsis thaliana] gi 836942 gb AAA67655; contains protein kinase domain, Pfam:PF00069; contains EF hand domain (calcium-binding EF-hand), Pfam:PF00036, INTERPRO:IPR002048 (evaluate: 1e-162, score=571.2) genbank/nr: gi 17064926 gb AAL32617.1 calcium-dependent protein kinase [Arabidopsis thaliana] gi 28059078 gb AAO29985.1 calcium-dependent protein kinase [Arabidopsis thaliana] (evaluate: 4e-161, score=571.2)	1.49	0.09
SGN-U212665	arabidopsis/peptide: At3g51850.1 68410.m05270 calcium-dependent protein kinase, putative (CDPK) similar to calcium-dependent protein kinase [Arabidopsis thaliana] gi 836942 gb AAA67655; contains protein kinase domain, Pfam:PF00069; contains EF hand domain (calcium-binding EF-hand), Pfam:PF00036, INTERPRO:IPR002048 (evaluate: 1e-162, score=571.2) genbank/nr: gi 17064926 gb AAL32617.1 calcium-dependent protein kinase [Arabidopsis thaliana] gi 28059078 gb AAO29985.1 calcium-dependent protein kinase [Arabidopsis thaliana] (evaluate: 4e-161, score=571.2)	1.55	0.06
SGN-U212854	arabidopsis/peptide: At3g43810.1 68410.m04339 calmodulin almost identical to calmodulin GI:16227 from [Arabidopsis thaliana] (evaluate: 6.3e-66, score=248.8) genbank/nr: gi 115525 sp P04353 CALM_SPIOL Calmodulin gi 71685 pir MCSP calmodulin - spinach (tentative sequence) (evaluate: 2.5e-64, score=248.8)	2.42	0.01
SGN-U213545	arabidopsis/peptide: At3g43810.1 68410.m04339 calmodulin almost identical to calmodulin GI:16227 from [Arabidopsis thaliana] (evaluate: 2.1e-76, score=282) genbank/nr: gi 115513 sp P27161 CALM_LYCES Calmodulin gi 170396 gb AAA34144.1 calmodulin gi 3549695 emb CAA09302.1 calmodulin 3 protein [Capsicum annum] gi 14625401 dbj BAB61907.1 calmodulin NtCaM1 [Nicotiana tabacum] gi 14625403 dbj BAB61908.1 calmodulin NtCaM2 [Nicotiana tabacum] gi 21616059 emb CAC84563.1 putative calmodulin [Solanum commersonii] (evaluate: 4.9e-79, score=295.8)	1.53	0.01

... continued ...

TABLE 1 Cont.

SGN-U214843	arabidopsis/peptide: At4g19110.2 68411.m05258 protein kinase, putative contains protein kinase domain, Pfam:PF00069 (evalue: 5e-171, score=598.6) genbank/nr: gi 30684655 ref NP_849407.1 protein kinase, putative [Arabidopsis thaliana] (evalue: 2e-169, score=598.6)	1.53	0.1
SGN-U215082	arabidopsis/peptide: At1g73500.1 68408.m07808 mitogen-activated protein kinase kinase (MAPKK), putative (MKK9) mitogen-activated protein kinase kinase (MAPKK) family, PMID:12119167 (evalue: 2e-101, score=366.3) genbank/nr: gi 15219482 ref NP_177492.1 mitogen-activated protein kinase kinase (MAPKK), putative (MKK9) [Arabidopsis thaliana] gi 25287619 pir G96761 probable MAP kinase T9L24.32 [imported] - Arabidopsis thaliana gi 11120804 gb AAG30984.1 MAP kinase, putative [Arabidopsis thaliana] gi 21536805 gb AAM61137.1 MAP kinase, putative [Arabidopsis thaliana] gi 26452087 dbj BAC43133.1 unknown protein [Arabidopsis thaliana] gi 28950881 gb AAO63364.1 At1g73500 [Arabidopsis thaliana] (evalue: 7e-100, score=366.3)	2.05	0.01
SGN-U215107	arabidopsis/peptide: At1g21410.1 68408.m02420 F-box protein family similar to SKP1 interacting partner 2 (SKIP2) TIGR_Ath1:At5g67250 (evalue: 1.2e-52, score=117.1) genbank/nr: gi 21554029 gb AAM63110.1 F-box protein AtFBL5 [Arabidopsis thaliana] (evalue: 3.8e-51, score=117.1)	1.56	0.03
SGN-U216024	arabidopsis/peptide: At5g48380.1 68412.m05390 leucine rich repeat protein family contains protein kinase domain, Pfam:PF00069; contains leucine-rich repeats, Pfam:PF00560 (evalue: 0, score=716.8) genbank/nr: gi 18422906 ref NP_568696.1 leucine rich repeat protein family [Arabidopsis thaliana] gi 13605827 gb AAK32899.1 AT5g48380/MJE7_1 [Arabidopsis thaliana] gi 18389278 gb AAL67082.1 putative receptor protein kinase [Arabidopsis thaliana] (evalue: 0, score=716.8)	1.87	0.1
SGN-U216378	arabidopsis/peptide: At3g52180.1 68410.m05308 expressed protein (evalue: 4e-134, score=474.9) genbank/nr: gi 14970762 emb CAC44460.1 protein tyrosine phosphatase [Lycopersicon esculentum] (evalue: 0, score=754.2)	1.52	0.1
SGN-U216696	arabidopsis/peptide: At4g28400.1 68411.m03704 protein phosphatase 2C (PP2C), putative protein phosphatase 2C-fission yeast, PIR2:S54297 (evalue: 3e-103, score=372.1) genbank/nr: gi 46277128 gb AAS86762.1 protein phosphatase 2C [Lycopersicon esculentum] (evalue: 9e-103, score=375.9)	1.65	0.02
SGN-U216698	arabidopsis/peptide: At1g13900.1 68408.m01475 calcineurin-like phosphoesterase family contains Pfam profile: PF00149 calcineurin-like phosphoesterase (evalue: 6e-101, score=364.8) genbank/nr: gi 15222978 ref NP_172843.1 calcineurin-like phosphoesterase family [Arabidopsis thaliana] gi 8778406 gb AAF79414.1 F16A14.11 [Arabidopsis thaliana] (evalue: 2.2e-99, score=364.8)	1.8	0.05

... continued ...

TABLE 1 Cont.

SGN-U220589	arabidopsis/peptide: At3g11410.1 68410.m01257 protein phosphatase 2C (PP2C), putative identical to protein phosphatase 2C (PP2C) GB:P49598 [Arabidopsis thaliana] (evalue: 5e-47, score=184.5) genbank/nr: gi 4336434 gb AAD17804.1 nodule-enhanced protein phosphatase type 2C [Lotus japonicus] (evalue: 8.4e-51, score=202.2)	1.95	0.04
SGN-U222721	arabidopsis/peptide: At4g28600.1 68411.m03727 calmodulin-binding protein similar to pollen-specific calmodulin-binding protein MPCBP GI:10086260 from [Zea mays] (evalue: 4.5e-84, score=308.1) genbank/nr: gi 22329002 ref NP_194589.2 calmodulin-binding protein [Arabidopsis thaliana] (evalue: 1.7e-82, score=308.1)	1.78	0.02
SGN-U223724	arabidopsis/peptide: At3g11410.1 68410.m01257 protein phosphatase 2C (PP2C), putative identical to protein phosphatase 2C (PP2C) GB:P49598 [Arabidopsis thaliana] (evalue: 7.5e-46, score=127.5) genbank/nr: gi 10432446 emb CAC10358.1 protein phosphatase 2C [Nicotiana tabacum] gi 22553023 emb CAC84141.2 protein phosphatase 2C [Nicotiana tabacum] (evalue: 1.3e-55, score=162.5)	1.46	0.02
SGN-U225762	arabidopsis/peptide: At5g43920.1 68412.m04843 transducin / WD-40 repeat protein family contains 7 WD-40 repeats (PF00400); similar to will die slowly protein (WDS) (SP:Q9V3J8) [Drosophila melanogaster] (evalue: 5.8e-72, score=267.3) genbank/nr: gi 15240036 ref NP_199205.1 transducin / WD-40 repeat protein family [Arabidopsis thaliana] gi 9758551 dbj BAB09052.1 WD-repeat protein-like [Arabidopsis thaliana] (evalue: 2.1e-70, score=267.3)	1.72	0.1
SGN-U226074	arabidopsis/peptide: At4g11655.1 68417.m01863 transmembrane protein, putative contains 4 transmembrane spanning domains, PMID:11152613 (evalue: 1e-28, score=124.4) genbank/nr: gi 42572877 ref NP_974535.1 transmembrane protein, putative [Arabidopsis thaliana] (evalue: 4e-27, score=124.4)	1.96	0.03
SGN-U226415	arabidopsis/peptide: At1g53430.1 68408.m05556 receptor-related serine/threonine kinase similar to receptor-like serine/threonine kinase GB:AAC50043 GI:2465923 from [Arabidopsis thaliana] (evalue: 1.8e-56, score=215.7) genbank/nr: gi 38228683 emb CAE54078.1 receptor-like protein kinase [Fagus sylvatica] (evalue: 8.6e-57, score=221.9)	1.97	0.02
SGN-U227357	arabidopsis/peptide: At1g71010.1 68408.m07513 expressed protein (evalue: 7.2e-55, score=124.4) genbank/nr: gi 42563125 ref NP_177257.3 phosphatidylinositol-4-phosphate 5-kinase family protein [Arabidopsis thaliana] gi 5902400 gb AAD55502.1 Unknown protein [Arabidopsis thaliana] (evalue: 2.7e-53, score=124.4)	2.23	0.02
SGN-U227773	arabidopsis/peptide: At1g49740.1 68408.m05109 expressed protein similar to MAP3K-like protein kinase GB:CAB16796 GI:4006878 from [Arabidopsis thaliana] (evalue: 1e-102, score=369.4) genbank/nr: gi 25407778 pir D85436 MAP3K-like protein kinase [imported] - Arabidopsis thaliana gi 4006878 emb CAB16796.1 MAP3K-like protein kinase [Arabidopsis thaliana] gi 7270644 emb CAB80361.1 MAP3K-like protein kinase [Arabidopsis thaliana] (evalue: 7e-105, score=382.1)	1.52	0.02

... continued ...

TABLE 1 Cont.

SGN-U230493	arabidopsis/peptide: At1g30440.1 68408.m03369 phototropic response protein family contains NPH3 family domain, Pfam:PF03000 (evalue: 4e-58, score=220.3) genbank/nr: gi 15220750 ref NP_174332.1 phototropic response protein family [Arabidopsis thaliana] (evalue: 1.2e-56, score=220.3)	1.75	0.01
STRESS RESPONSE			
SGN-U214993	arabidopsis/peptide: At5g02380.1 68412.m00149 metallothionein 2b (evalue: 4.3e-07, score=51.22) genbank/nr: gi 2497896 sp Q40157 MT2A_LYCES Metallothionein-like protein type 2 A (LeMT(A)) gi 7441812 pir T07073 metallothionein type II A - tomato gi 1449136 gb AAB04674.1 metallothionein II-like protein [Lycopersicon esculentum] (evalue: 1.2e-23, score=111.3)	1.48	0.04
SGN-U216624	arabidopsis/peptide: At1g61770.1 68408.m06387 DnaJ protein family similar to SP Q9UBS4 DnaJ homolog subfamily B member 11 precursor Homo sapiens; contains Pfam profile PF00226 DnaJ domain (evalue: 4e-118, score=421.8) genbank/nr: gi 30696610 ref NP_176370.2 DnaJ protein family [Arabidopsis thaliana] gi 26983836 gb AAN86170.1 unknown protein [Arabidopsis thaliana] (evalue: 1e-116, score=421.8)	1.46	0.04
SGN-U221325	arabidopsis/peptide: At4g28480.1 68411.m03712 heat shock protein, putative similar to SP Q9UDY4 DJB4_HUMAN DnaJ homolog subfamily B member 4 (Heat shock 40 kDa protein 1 homolog) {Homo sapiens}; contains Pfam profile PF00226: DnaJ domain (evalue: 1.1e-58, score=223.8) genbank/nr: gi 15235310 ref NP_194577.1 heat shock protein, putative [Arabidopsis thaliana] gi 7441938 pir T04618 heat shock protein homolog F2009.160 - Arabidopsis thaliana gi 2842490 emb CAA16887.1 heat-shock protein [Arabidopsis thaliana] gi 7269702 emb CAB79650.1 heat-shock protein [Arabidopsis thaliana] gi 14596115 gb AAK68785.1 heat-shock protein [Arabidopsis thaliana] gi 20148389 gb AAM10085.1 heat-shock protein [Arabidopsis thaliana] (evalue: 4e-57, score=223.8)	1.84	0.04
SGN-U221384	arabidopsis/peptide: At1g71950.1 68408.m07632 expressed protein similar to Pi starvation-induced protein GB:BAA06151 from [Nicotiana tabacum] (evalue: 8.5e-31, score=130.6) genbank/nr: gi 7489176 pir T03677 pit2 protein (clone pAL141), Pi starvation induced - common tobacco gi 676884 dbj BAA06151.1 The expression is induced by Pi starvation. [Nicotiana tabacum] gi 1094819 prf 2106387C AI-induced protein (evalue: 2e-36, score=154.5)	1.43	0.01
TRANSCRIPTION FACTOR			
SGN-U213896	arabidopsis/peptide: At3g01470.1 68410.m00060 homeobox-leucine zipper protein HAT5 (HD-ZIP protein 5) (HD-ZIP protein ATHB-1) identical to homeobox-leucine zipper protein HAT5 (HD-ZIP protein 5) (HD-ZIP protein ATHB-1) GB:Q02283 [Arabidopsis thaliana] (evalue: 5.9e-38, score=155.6) genbank/nr: gi 15148918 gb AAK84886.1 homeodomain leucine zipper protein	1.44	0.09

... continued ...

TABLE 1 Cont.

	HDZ2 [<i>Phaseolus vulgaris</i>] (evalue: 4.2e-78, score=294.3)		
SGN-U214021	arabidopsis/peptide: At1g69780.1 68408.m07365 homeobox-leucine zipper protein ATHB-13 (HD-Zip transcription factor Athb-13) identical to homeobox gene 13 protein (GP:12325190) [<i>Arabidopsis thaliana</i>] (evalue: 7.4e-99, score=357.8) genbank/nr: gi 48057565 gb AAT39931.1 putative HD-zip protein [<i>Solanum demissum</i>] (evalue: 4e-174, score=613.2)	1.4	0.06
SGN-U214635	arabidopsis/peptide: At2g35940.2 68409.m05699 homeodomain protein contains 'Homeobox' domain signature, Prosite:PS00027 (evalue: 3.4e-83, score=306.2) genbank/nr: gi 31323447 gb AAP47025.1 bell-like homeodomain protein 2 [<i>Lycopersicon esculentum</i>] (evalue: 0, score=729.6)	2.54	0.07
SGN-U214896	arabidopsis/peptide: At2g41900.1 68409.m04689 CCCH-type zinc finger protein -related also an ankyrin-repeat protein (evalue: 0, score=667.5) genbank/nr: gi 28273376 gb AAO38462.1 unknown prot [<i>Oryza sativa</i> (japonica cultivar-group)] (evalue: 0, score=674.5)	2	0.05
SGN-U215777	arabidopsis/peptide: At3g55770.1 68410.m05721 transcription factor L2 (evalue: 8.9e-93, score=337) genbank/nr: gi 7489184 pir T03400 probable transcription factor SF3 - common tobacco gi 1841464 emb CAA71891.1 LIM-domain SF3 protein [<i>Nicotiana tabacum</i>] gi 5932420 gb AAD56951.1 LIM domain protein WLIM2 [<i>Nicotiana tabacum</i>] (evalue: 2e-103, score=377.9)	1.52	0.01
SGN-U216582	arabidopsis/peptide: At4g16420.1 68411.m02261 transcriptional adaptor like protein (evalue: 6.5e-23, score=104.8) genbank/nr: gi 18414653 ref NP_567495.1 transcriptional adaptor like protein [<i>Arabidopsis thaliana</i>] gi 13591700 gb AAK31320.1 transcriptional adaptor ADA2b [<i>Arabidopsis thaliana</i>] gi 15215640 gb AAK91365.1 AT4g16420/dl4235c [<i>Arabidopsis thaliana</i>] gi 23505981 gb AAN28850.1 At4g16420/dl4235c [<i>Arabidopsis thaliana</i>] (evalue: 2.4e-21, score=104.8)	2.56	0.02
SGN-U216849	arabidopsis/peptide: At5g62000.3 68418.m07784 transcriptional factor B3 family protein / auxin-responsive factor, putative (ARF1) contains Pfam profile: PF02362 B3 DNA binding domain; identical to cDNA ARF1 (auxin response factor) binding protein GI:2245393 (evalue: 4e-167, score=565.5) genbank/nr: gi 30027167 gb AAP06759.1 auxin response factor-like protein [<i>Mangifera indica</i>] (evalue: 2e-169, score=580.5)	1.73	0.04
SGN-U217929	arabidopsis/peptide: At3g60800.1 68410.m06284 DHHC-type zinc finger domain-containing protein contains DHHC zinc finger domain PF01529 (evalue: 2.2e-72, score=268.9) genbank/nr: gi 22331887 ref NP_191639.2 DHHC-type zinc finger domain-containing protein [<i>Arabidopsis thaliana</i>] gi 17979303 gb AAL49877.1 unknown protein [<i>Arabidopsis thaliana</i>] gi 20466005 gb AAM20224.1 unknown protein [<i>Arabidopsis thaliana</i>] (evalue: 7.9e-71, score=268.9)	1.57	0.09

... continued ...

TABLE 1 Cont.

SGN-U219383	arabidopsis/peptide: At1g10200.1 68408.m01037 transcription factor - related similar to transcription factor SF3 (pir IS37656); similar to ESTs gb T42207, gb N37716, and emb Z17491 (evaluate: 7.2e-74, score=274.2) genbank/nr: gi 5932418 gb AAD56950.1 LIM domain protein PLIM1 [Nicotiana tabacum] (evaluate: 7.7e-75, score=282.7)	1.7	0.06
SGN-U221901	arabidopsis/peptide: At1g26580.1 68408.m02934 expressed protein similar to putative MYB family transcription factor GB:AAD17429 GI:4335752 from [Arabidopsis thaliana] (evaluate: 3.2e-11, score=66.24) genbank/nr: gi 15222736 ref NP_173980.1 expressed protein [Arabidopsis thaliana] gi 25518777 pir H86392 hypothetical protein T1K7.5 - Arabidopsis thaliana gi 9797742 gb AAF98560.1 Contains similarity to a putative MYB family transcription factor gene T4M8.10 gi 4335752 from Arabidopsis thaliana BAC T4M8 gb AC006284 and contains a Myb-like DNA-binding PF 00249 domain. ESTs gb T75914, gb T45901 come from this gene (evaluate: 1.2e-09, score=66.24)	1.63	0.09
SGN-U222328	arabidopsis/peptide: At5g13790.1 68412.m01484 floral homeotic protein, AGL15 (evaluate: 8.2e-51, score=198) genbank/nr: gi 34452081 gb AAQ72497.1 MADS-box protein 9 [Petunia x hybrida] (evaluate: 7e-107, score=389.4)	1.59	0.06
SGN-U222814	arabidopsis/peptide: At3g46640.1 68410.m04691 myb family transcription factor contains Pfam profile: PF00249 myb-like DNA-binding domain (evaluate: 1.5e-16, score=83.57) genbank/nr: gi 15232597 ref NP_190248.1 myb family transcription factor [Arabidopsis thaliana] gi 11357354 pir T45601 hypothetical protein F12A12.160 - Arabidopsis thaliana gi 6523067 emb CAB62334.1 putative protein [Arabidopsis thaliana] gi 30102630 gb AAP21233.1 At3g46640 [Arabidopsis thaliana] (evaluate: 5.4e-15, score=83.57)	1.99	0.09
SGN-U223527	arabidopsis/peptide: At1g77980.1 68414.m09087 MADS-box family protein MADS-box protein AGL66 (evaluate: 1.5e-13, score=72.4) genbank/nr: gi 42408790 dbj BAD10025.1 putative MADS-box protein [Oryza sativa (japonica cultivar-group)] >gi 42408843 dbj BAD10102.1 putative MADS-box protein [Oryza sativa (japonica cultivar-group)] (evaluate: 9.5e-15, score=81.26)	1.54	0.05
SGN-U228962	arabidopsis/peptide: At2g38250.1 68409.m04249 GT-1-related transcription factor (evaluate: 9.9e-25, score=110.5) genbank/nr: gi 38344145 emb CAD41865.2 OSJNBa0041A02.12 [Oryza sativa (japonica cultivar-group)] (evaluate: 6.7e-38, score=159.5)	2	0.03
SGN-U230858	arabidopsis/peptide: At2g40620.1 68409.m04539 bZip DNA binding protein identical to b-Zip DNA binding protein GI:2246376 from [Arabidopsis thaliana]; contains a bZIP transcription factor basic domain signature (PDOC00036) (evaluate: 2.3e-31, score=132.5) genbank/nr: gi 15226727 ref NP_181594.1 bZip DNA binding protein [Arabidopsis thaliana] gi 25408715 pir G84831 probable bZIP transcription factor [imported] - Arabidopsis thaliana gi 2651296 gb AAB87576.1 putative bZIP transcription factor [Arabidopsis thaliana] gi 18377632 gb AAL66966.1 putative bZIP transcription factor [Arabidopsis thaliana]	1.42	0

... continued ...

TABLE 1 Cont.

	gi 20465783 gb AAM20380.1 putative bZIP transcription factor [Arabidopsis thaliana] (evalue: 8.3e-30, score=132.5)		
SGN-U231468	arabidopsis/peptide: At3g60800.1 68410.m06284 DHHC-type zinc finger domain-containing protein contains DHHC zinc finger domain PF01529 (evalue: 1e-36, score=149.8) genbank/nr: gi 11358343 pir T47891 hypothetical protein T4C21.210 - Arabidopsis thaliana gi 7329690 emb CAB82684.1 putative protein [Arabidopsis thaliana] (evalue: 3.4e-35, score=149.8)	1.52	0.05
TRANSPORT			
SGN-U214683	arabidopsis/peptide: At5g62670.1 68412.m07112 ATPase, plasma membrane-type (proton pump), putative strong similarity to P-type H(+)-transporting ATPase from Nicotiana plumbaginifolia [SP Q08435, SP Q08436], Lycopersicon esculentum [GI:5901757, SP P22180], Solanum tuberosum [GI:435003]; contains InterPro accession IPR001757: ATPase, E1-E2 type (evalue: 0, score=710.3) genbank/nr: gi 25290692 pir T52412 H+-exporting ATPase (EC 3.6.3.6) plasma membrane isoform LHA2 [imported] - tomato gi 5901757 gb AAD55399.1 plasma membrane H+-ATPase isoform LHA2 [Lycopersicon esculentum] gi 9789539 gb AAF98344.1 plasma membrane H+-ATPase [Lycopersicon esculentum] (evalue: 0, score=760)	1.58	0
SGN-U215141	arabidopsis/peptide: At4g09650.1 68411.m01429 H+-transporting ATP synthase-related protein H+-transporting ATP synthase (EC 3.6.1.34) delta chain precursor, chloroplast - Nicotiana tabacum, PIR2:S26198 (evalue: 5.4e-68, score=254.6) genbank/nr: gi 416681 sp P32980 ATPD_TOBAC ATP synthase delta chain, chloroplast precursor gi 280404 pir S26198 H+-transporting two-sector ATPase (EC 3.6.3.14) delta chain precursor, chloroplast - common tobacco gi 19787 emb CAA45153.1 chloroplast ATP synthase (delta subunit) [Nicotiana tabacum] (evalue: 9e-102, score=372.1)	1.53	0.01
SGN-U216732	arabidopsis/peptide: At5g09260.1 68412.m00988 hypothetical protein SNF7 protein - Saccharomyces cerevisiae, PIR:S52590 (evalue: 7.3e-43, score=170.2) genbank/nr: gi 15242368 ref NP_196488.1 hypothetical protein [Arabidopsis thaliana] gi 9955513 emb CAC05452.1 putative protein [Arabidopsis thaliana] gi 34365667 gb AAQ65145.1 At5g09260 [Arabidopsis thaliana] (evalue: 2.5e-41, score=170.2)	1.47	0
SGN-U217510	arabidopsis/peptide: At1g14360.1 68408.m01539 expressed protein (evalue: 7.4e-62, score=232.6) genbank/nr: gi 48209877 gb AAT40483.1 putative UDP-galactose transporter [Solanum demissum] (evalue: 5e-65, score=248.1)	1.52	0.02
SGN-U225750	arabidopsis/peptide: At4g35300.1 68411.m05407 transporter - related low similarity to hexose transporter [Solanum tuberosum] GI:8347246; contains Pfam profile PF00083: major facilitator superfamily protein (evalue: 4.1e-44, score=174.9) genbank/nr: gi 26986186 emb CAD58958.1 hexose transporter [Hordeum vulgare subsp. vulgare] (evalue: 7.1e-45, score=182.6)	1.87	0

... continued ...

TABLE 1 Cont.

UNKNOWN				
SGN-U213029	arabidopsis/peptide: At3g17860.1 68410.m02052 expressed protein (evalue: 1.2e-26, score=117.1) genbank/nr: gi 39652276 dbj BAD04851.1 hypothetical protein [Solanum tuberosum] (evalue: 2.1e-25, score=118.2)	1.4	0.09	
SGN-U213465	arabidopsis/peptide: At1g69230.2 68414.m07930 expressed protein (evalue: 1.3e-31, score=132.9) genbank/nr: gi 25518436 pir C86390 hypothetical protein T1K7.26 - Arabidopsis thaliana gi 9797761 gb AAF98579.1 Contains similarity to PIR7A protein from Oryza sativa gb Z34271 and contains an alpha/beta hydrolase fold PF 00561. [Arabidopsis thaliana] (evalue: 2.1e-32, score=140.6)	1.74	0.03	
SGN-U213550	arabidopsis/peptide: At4g11220.1 68411.m01643 expressed protein 24 kDa seed maturation protein - Glycine max,PID:g4102690 (evalue: 1.3e-82, score=303.5) genbank/nr: gi 34909318 ref NP_916006.1 OSJNBb0021A09.5 [Oryza sativa (japonica cultivar-group)] gi 20160502 dbj BAB89453.1 OSJNBb0021A09.5 [Oryza sativa (japonica cultivar-group)] (evalue: 8.7e-83, score=309.3)	1.46	0.07	
SGN-U214640	arabidopsis/peptide: At1g20580.1 68408.m02319 expressed protein (evalue: 4.7e-50, score=194.5) genbank/nr: gi 18394883 ref NP_564119.1 expressed protein [Arabidopsis thaliana] gi 8886923 gb AAF80609.1 F2D10.7 [Arabidopsis thaliana] gi 15724292 gb AAL06539.1 At1g20580/F2D10_6 [Arabidopsis thaliana] gi 20334748 gb AAM16235.1 At1g20580/F2D10_6 [Arabidopsis thaliana] (evalue: 1.7e-48, score=194.5)	1.48	0.09	
SGN-U214730	arabidopsis/peptide: At4g04955.1 68411.m00631 expressed protein (evalue: 0, score=691) genbank/nr: gi 18412757 ref NP_567276.1 expressed protein [Arabidopsis thaliana] gi 15028089 gb AAK76575.1 unknown protein [Arabidopsis thaliana] gi 21281139 gb AAM44996.1 unknown protein [Arabidopsis thaliana] (evalue: 0, score=691)	2.13	0.06	
SGN-U214813	arabidopsis/peptide: At5g23530.1 68412.m02530 expressed protein contains similarity to PrMC3 [Pinus radiata] GI:5487873 (evalue: 1e-112, score=403.3) genbank/nr: gi 15237783 ref NP_197744.1 expressed protein [Arabidopsis thaliana] gi 8809707 dbj BAA97248.1 contains similarity to unknown protein~gb AAF27018.1~gene_id:MQM1.21 [Arabidopsis thaliana] (evalue: 5e-111, score=403.3)	2.05	0.09	
SGN-U215491	arabidopsis/peptide: At1g55160.1 68408.m05774 expressed protein (evalue: 5.9e-25, score=110.5) genbank/nr: gi 18405239 ref NP_564677.1 expressed protein [Arabidopsis thaliana] gi 25405797 pir C96593 unknown protein, 99945-98618 [imported] - Arabidopsis thaliana gi 12321579 gb AAG50842.1 unknown protein [Arabidopsis thaliana] gi 12323174 gb AAG51570.1 unknown protein; 99945-98618 [Arabidopsis thaliana] gi 13937163 gb AAK50075.1 At1g55160/T7N22.11 [Arabidopsis thaliana] gi 22137148 gb AAM91419.1 At1g55160/T7N22.11 [Arabidopsis thaliana] (evalue: 1.9e-23, score=110.5)	2.15	0.01	

... continued ...

TABLE 1 Cont.

SGN-U215613	arabidopsis/peptide: At1g02475.1 68408.m00181 expressed protein (evalue: 2.8e-66, score=249.2) genbank/nr: gi 18378973 ref NP_563656.1 expressed protein [Arabidopsis thaliana] gi 13878059 gb AAK44107.1 unknown protein [Arabidopsis thaliana] gi 17104651 gb AAL34214.1 unknown protein [Arabidopsis thaliana] (evalue: 1.1e-64, score=249.2)	2.2	0.03
SGN-U215870	arabidopsis/peptide: At1g03250.1 68408.m00271 expressed protein EST gb N96383 comes from this gene (evalue: 2.8e-73, score=272.3) genbank/nr: gi 18379075 ref NP_563680.1 expressed protein [Arabidopsis thaliana] gi 21593601 gb AAM65568.1 unknown [Arabidopsis thaliana] gi 22022560 gb AAM83237.1 At1g03250/F15K9_13 [Arabidopsis thaliana] gi 23308263 gb AAN18101.1 At1g03250/F15K9_13 [Arabidopsis thaliana] (evalue: 1e-71, score=272.3)	1.7	0.01
SGN-U216025	arabidopsis/peptide: At5g19300.1 68412.m02105 hypothetical protein predicted proteins, H. sapiens, D. melanogaster and others (evalue: 2e-120, score=429.5) genbank/nr: gi 42567956 ref NP_197431.2 expressed protein [Arabidopsis thaliana] >gi 45825145 gb AAS77480.1 At5g19300 [Arabidopsis thaliana] (evalue: 8e-119, score=429.5)	1.82	0.02
SGN-U216096	No hits found	1.71	0.04
SGN-U216110	arabidopsis/peptide: At5g51010.1 68412.m05714 expressed protein (evalue: 2.9e-38, score=156) genbank/nr: gi 18423226 ref NP_568749.1 expressed protein [Arabidopsis thaliana] gi 9758248 dbj BAB08747.1 gene_id:K3K7.19~unknown protein [Arabidopsis thaliana] gi 15292669 gb AAK92703.1 unknown protein [Arabidopsis thaliana] gi 19310697 gb AAL85079.1 unknown protein [Arabidopsis thaliana] gi 21555410 gb AAM63852.1 unknown [Arabidopsis thaliana] (evalue: 1.1e-36, score=156)	1.48	0.06
SGN-U216215	arabidopsis/peptide: At1g07080.1 68408.m00686 expressed protein (evalue: 1.6e-74, score=276.6) genbank/nr: gi 25406953 pir F86205 hypothetical protein [imported] - Arabidopsis thaliana gi 8954038 gb AAF82212.1 Contains similarity to an unknown protein F7A7_100 gi 7327817 from Arabidopsis thaliana BAC F7A7 gb AL161946. ESTs gb N65842, gb F19836 and gb AI993679 come from this gene (evalue: 6.2e-73, score=276.6)	1.69	0.02
SGN-U217127	arabidopsis/peptide: At5g04080.1 68412.m00365 expressed protein (evalue: 2.8e-12, score=68.94) genbank/nr: gi 30680382 ref NP_196028.2 expressed protein [Arabidopsis thaliana] (evalue: 9.7e-11, score=68.94)	1.63	0.06
SGN-U217198	arabidopsis/peptide: At5g08050.1 68412.m00872 expressed protein predicted protein, Arabidopsis thaliana (evalue: 8.6e-33, score=136.7) genbank/nr: gi 17978981 gb AAL47451.1 AT5g08050/F13G24_250 [Arabidopsis thaliana] gi 33589718 gb AAQ22625.1 At5g08050/F13G24_250 [Arabidopsis thaliana] (evalue: 2.9e-31, score=136.7)	1.77	0.02

... continued ...

TABLE 1 Cont.

SGN-U217288	arabidopsis/peptide: At2g03420.1 68409.m00267 expressed protein (evalue: 4.7e-46, score=181.4) genbank/nr: gi 18395549 ref NP_565301.1 expressed protein [Arabidopsis thaliana] gi 20197739 gb AAD17434.2 hypothetical protein [Arabidopsis thaliana] gi 26450217 dbj BAC42227.1 unknown protein [Arabidopsis thaliana] gi 28827508 gb AAO50598.1 unknown protein [Arabidopsis thaliana] (evalue: 1.7e-44, score=181.4)	1.77	0.1
SGN-U217312	arabidopsis/peptide: At1g11760.1 68408.m01217 expressed protein (evalue: 1.6e-44, score=176.4) genbank/nr: gi 28466841 gb AAO44029.1 At1g11760 [Arabidopsis thaliana] (evalue: 5.2e-47, score=189.9)	1.48	0.02
SGN-U217758	arabidopsis/peptide: At2g35880.1 68409.m03986 expressed protein (evalue: 2.2e-55, score=213.8) genbank/nr: gi 18403980 ref NP_565829.1 expressed protein [Arabidopsis thaliana] gi 16209720 gb AAL14415.1 At2g35880/F11F19.21 [Arabidopsis thaliana] gi 20197995 gb AAD21469.2 expressed protein [Arabidopsis thaliana] gi 22655282 gb AAM98231.1 unknown protein [Arabidopsis thaliana] (evalue: 8.6e-54, score=213.8)	1.63	0.07
SGN-U217844	arabidopsis/peptide: At3g07568.1 68410.m00823 expressed protein (evalue: 9e-09, score=57) genbank/nr: gi 30680359 ref NP_850535.1 expressed protein [Arabidopsis thaliana] gi 17065402 gb AAL32855.1 Unknown protein [Arabidopsis thaliana] gi 20148611 gb AAM10196.1 unknown protein [Arabidopsis thaliana] gi 21554046 gb AAM63127.1 unknown [Arabidopsis thaliana] (evalue: 3.1e-07, score=57)	1.69	0.01
SGN-U218009	arabidopsis/peptide: At5g19855.1 68412.m02163 expressed protein (evalue: 2.5e-60, score=229.2) genbank/nr: gi 18420050 ref NP_568382.1 expressed protein [Arabidopsis thaliana] gi 21593746 gb AAM65713.1 unknown [Arabidopsis thaliana] gi 28393243 gb AAO42050.1 unknown protein [Arabidopsis thaliana] gi 28827676 gb AAO50682.1 unknown protein [Arabidopsis thaliana] (evalue: 9.6e-59, score=229.2)	1.97	0.01
SGN-U218047	arabidopsis/peptide: At1g29700.1 68408.m03287 expressed protein (evalue: 2e-105, score=379.4) genbank/nr: gi 18397206 ref NP_564334.1 expressed protein [Arabidopsis thaliana] gi 25403086 pir D86420 unknown protein [imported] - Arabidopsis thaliana gi 12321412 gb AAG50777.1 unknown protein [Arabidopsis thaliana] gi 12323515 gb AAG51727.1 unknown protein; 129333-127623 [Arabidopsis thaliana] gi 14596083 gb AAK68769.1 Unknown protein [Arabidopsis thaliana] gi 18377530 gb AAL66931.1 unknown protein [Arabidopsis thaliana] (evalue: 7e-104, score=379.4)	1.59	0.04
SGN-U218081	arabidopsis/peptide: At2g39950.1 68409.m04448 expressed protein and gencan (evalue: 3e-46, score=181.4) genbank/nr: gi 15225598 ref NP_181524.1 expressed protein [Arabidopsis thaliana] gi 25408647 pir D84823 hypothetical protein At2g39950 [imported] - Arabidopsis thaliana gi 2088648 gb AAB95280.1 hypothetical protein [Arabidopsis thaliana] (evalue: 1e-44, score=181.4)	1.68	0.09

... continued ...

TABLE 1 Cont.

SGN-U218211	arabidopsis/peptide: At2g33845.1 68409.m03754 expressed protein (evalue: 1.5e-39, score=159.8) genbank/nr: gi 18403397 ref NP_565774.1 expressed protein [Arabidopsis thaliana] gi 20198312 gb AAM15519.1 Expressed protein [Arabidopsis thaliana] gi 21592737 gb AAM64686.1 unknown [Arabidopsis thaliana] gi 22530954 gb AAM96981.1 expressed protein [Arabidopsis thaliana] gi 23198430 gb AAN15742.1 expressed protein [Arabidopsis thaliana] (evalue: 5.5e-38, score=159.8)	1.61	0.01
SGN-U218673	arabidopsis/peptide: At4g00370.1 68411.m00049 expressed protein (evalue: 0, score=640.2) genbank/nr: gi 30678625 ref NP_567175.2 expressed protein [Arabidopsis thaliana] gi 26451814 dbj BAC43000.1 unknown protein [Arabidopsis thaliana] gi 32306495 gb AAP78931.1 At4g00370 [Arabidopsis thaliana] (evalue: 0, score=640.2)	1.57	0.02
SGN-U218850	arabidopsis/peptide: At1g62310.1 68408.m06446 expressed protein (evalue: 1.4e-63, score=147.1) genbank/nr: gi 15220761 ref NP_176421.1 expressed protein [Arabidopsis thaliana] (evalue: 5.7e-62, score=147.1)	1.8	0.04
SGN-U219158	arabidopsis/peptide: At1g02070.1 68408.m00117 expressed protein (evalue: 1.2e-10, score=63.54) genbank/nr: gi 46806700 dbj BAD17770.1 hypothetical protein [Oryza sativa (japonica cultivar-group)] >gi 46806728 dbj BAD17778.1 hypothetical protein [Oryza sativa (japonica cultivar-group)] (evalue: 3.7e-16, score=87.04)	3.09	0.03
SGN-U219500	arabidopsis/peptide: At2g31560.2 68415.m03856 expressed protein (evalue: 5.1e-48, score=188.3) genbank/nr: gi 30684991 ref NP_850169.1 expressed protein [Arabidopsis thaliana] gi 17528942 gb AAL38681.1 unknown protein [Arabidopsis thaliana] (evalue: 1.9e-46, score=188.3)	1.78	0.01
SGN-U219563	arabidopsis/peptide: At3g10260.2 68410.m06636 expressed protein similar to unknown protein GB:AAC62889 [Arabidopsis thaliana] (evalue: 2.3e-77, score=285.8) genbank/nr: gi 30681227 ref NP_850552.1 expressed protein [Arabidopsis thaliana] (evalue: 8.8e-76, score=285.8)	1.56	0.03
SGN-U220069	arabidopsis/peptide: At3g56880.1 68410.m05844 expressed protein predicted protein At2g41010 - Arabidopsis thaliana, EMBL:AC004261 (evalue: 1.7e-20, score=97.06) genbank/nr: gi 15230149 ref NP_191247.1 expressed protein [Arabidopsis thaliana] gi 11289614 pir T51276 hypothetical protein T8M16_210 - Arabidopsis thaliana gi 9663007 emb CAC00751.1 putative protein [Arabidopsis thaliana] gi 15028355 gb AAK76654.1 unknown protein [Arabidopsis thaliana] gi 24030447 gb AAN41377.1 unknown protein [Arabidopsis thaliana] (evalue: 6.4e-19, score=97.06)	1.43	0.05
SGN-U222203	arabidopsis/peptide: At2g27830.1 68409.m03055 expressed protein (evalue: 2.4e-37, score=152.5) genbank/nr: gi 10279678 emb CAC09928.1 hypothetical protein [Catharanthus roseus] (evalue: 3.8e-47, score=190.3)	1.59	0.01
SGN-U222620	No hits found	3.76	0.04

... continued ...

TABLE 1 Cont.

SGN-U222946	No hits found	1.51	0.1
SGN-U222946	No hits found	1.54	0.03
SGN-U223214	arabidopsis/peptide: At3g15050.1 68410.m01708 expressed protein similar to SF16 protein GB:CAA52782 [Helianthus annuus] (evaluate: 9.5e-56, score=214.5) genbank/nr: gi 15232474 ref NP_188123.1 expressed protein [Arabidopsis thaliana] gi 8777488 dbj BAA97068.1 contains similarity to SF16 protein~gene_id:K15M2.19 [Arabidopsis thaliana] (evaluate: 3.7e-54, score=214.5)	1.68	0.09
SGN-U223235	arabidopsis/peptide: At2g02050.1 68409.m00120 expressed protein (evaluate: 5.2e-41, score=163.7) genbank/nr: gi 18395290 ref NP_565280.1 expressed protein [Arabidopsis thaliana] gi 25410957 pir D84432 hypothetical protein At2g02050 [imported] - Arabidopsis thaliana gi 4406787 gb AAD20097.1 expressed protein [Arabidopsis thaliana] gi 11692816 gb AAG40011.1 At2g02050 [Arabidopsis thaliana] gi 11908122 gb AAG41490.1 unknown protein [Arabidopsis thaliana] gi 12642932 gb AAK00408.1 unknown protein [Arabidopsis thaliana] gi 15529288 gb AAK97738.1 At2g02050/F14H20.12 [Arabidopsis thaliana] gi 21593473 gb AAM65440.1 unknown [Arabidopsis thaliana] gi 23505757 gb AAN28738.1 At2g02050/F14H20.12 [Arabidopsis thaliana] (evaluate: 1.7e-39, score=163.7)	1.64	0.04
SGN-U223256	arabidopsis/peptide: At5g59080.1 68412.m06698 expressed protein (evaluate: 4.2e-11, score=65.47) genbank/nr: gi 15237814 ref NP_200716.1 expressed protein [Arabidopsis thaliana] gi 10177635 dbj BAB10783.1 emb CAB82975.1~gene_id:K18B18.8~similar to unknown protein [Arabidopsis thaliana] gi 17380960 gb AAL36292.1 unknown protein [Arabidopsis thaliana] gi 20465393 gb AAM20121.1 unknown protein [Arabidopsis thaliana] (evaluate: 1.6e-09, score=65.47)	1.53	0.06
SGN-U225377	No hits found	1.83	0.01
SGN-U228094	No hits found	2.17	0.06
SGN-U230225	arabidopsis/peptide: At5g67480.1 68412.m07705 expressed protein strong similarity to unknown protein (pir T04718) (evaluate: 2e-43, score=172.2) genbank/nr: gi 15240763 ref NP_201549.1 expressed protein [Arabidopsis thaliana] gi 9757869 dbj BAB08456.1 gene_id:K9I9.4~pir T04718~strong similarity to unknown protein [Arabidopsis thaliana] gi 15529178 gb AAK97683.1 AT5g67480/K9I9_4 [Arabidopsis thaliana] gi 17386120 gb AAL38606.1 AT5g67480/K9I9_4 [Arabidopsis thaliana] (evaluate: 6.8e-42, score=172.2)	1.53	0.01

... continued ...

TABLE 1 Cont.

SGN- U231560	arabidopsis/peptide: At4g27540.1 68411.m03603 expressed protein (evalue: 6.7e-14, score=73.94) genbank/nr: gi 18417012 ref NP_567776.1 expressed protein [Arabidopsis thaliana] gi 21536961 gb AAM61302.1 unknown [Arabidopsis thaliana] (evalue: 2.2e-12, score=73.94)	1.98	0.03
SGN- U232066	No hits found	1.83	0.09
SGN- U232089	No hits found	2.28	0.02
SGN- U232169	No hits found	2.29	0.1
SGN- U234375	No hits found	1.47	0
SGN- U234755	No hits found	2.03	0.08
SGN- U236156	No hits found	1.74	0.04
SGN- U237464	No hits found	1.47	0.05
SGN- U240428	No hits found	1.49	0.1
SGN- U240837	No hits found	2.04	0.01
SGN- U241296	No hits found	2.71	0.05

... continued ...

TABLE 1 Cont.

ID	Gene Annotation	Average of ratios	Pvalue
DOWN-REGULATED GENES			
CELL STRUCTURE			
SGN-U213235	arabidopsis/peptide: At5g65360.1 68412.m07436 histone H3 identical to histone H3 from Zea mays SP P05203, Medicago sativa GI:166384, Encephalartos altensteinii SP P08903, Pisum sativum SP P02300; contains Pfam profile PF00125 Core histone H2A/H2B/H3/H4 (evalue: 2.9e-56, score=214.9) genbank/nr: gi 15232146 ref NP_189372.1 histone H3 [Arabidopsis thaliana] gi 15238433 ref NP_201339.1 histone H3 [Arabidopsis thaliana] H3 histone [Nicotiana tabacum] gi 27764990 gb AAO23616.1 At5g10400 [Arabidopsis thaliana] gi 27808628 gb AAO24594.1 At1g09200 [Arabidopsis thaliana] gi 28973783 gb AAO64207.1 putative histone H3 [Arabidopsis thaliana] gi 29824185 gb AAP04053.1 putative histone H3 [Arabidopsis thaliana] gi 38345293 emb CAE02917.2 OSJNBb0108J11.9 [Oryza sativa (japonica cultivar-group)] gi 225459 prf 1303352A histone H3 gi 225839 prf 1314298B histone H3 (evalue: 1e-54, score=214.9)	0.58	0.06
SGN-U215583	arabidopsis/peptide: At1g07790.1 68408.m00763 histone H2B, putative strong similarity to histone H2B Arabidopsis thaliana GI:2407802, Gossypium hirsutum SP O22582, Lycopersicon esculentum GI:3021489, Capsicum annuum SP O49118; contains Pfam profile PF00125 Core histone H2A/H2B/H3/H4 (evalue: 2e-44, score=175.6) genbank/nr: gi 7387727 sp O49118 H2B_CAPAN Histone H2B (CaH2B) gi 7439757 pir T08063 histone H2B - pepper gi 2746719 gb AAB94923.1 histone H2B [Capsicum annuum] (evalue: 5.4e-43, score=176)	0.6	0.01
SGN-U214148	arabidopsis/peptide: At2g30620.1 68409.m03380 histone H1 (evalue: 1.1e-18, score=91.28) genbank/nr: gi 585241 sp P37218 H1_LYCES HISTONE H1 gi 629668 pir S45662 histone H1 - tomato gi 424100 gb AAA50578.1 histone H1 (evalue: 6e-32, score=140.6)	0.6	0.01

... continued ...

TABLE 1 Cont.

CELL WALL			
SGN-U231117	arabidopsis/peptide: At5g15050.1 68412.m01629 N-acetylglucosaminyltransferase family (Core-2/I-Branching enzyme family) contains Pfam profile: PF02485 Core-2/I-Branching enzyme (evalue: 1.2e-25, score=112.8) genbank/nr: gi 15242199 ref NP_197009.1 N-acetylglucosaminyltransferase family (Core-2/I-Branching enzyme family) [Arabidopsis thaliana] gi 11291967 pir T51450 hypothetical protein F2G14_170 - Arabidopsis thaliana gi 9755672 emb CAC01824.1 putative protein [Arabidopsis thaliana] gi 16209674 gb AAL14395.1 AT5g15050/F2G14_170 [Arabidopsis thaliana] gi 21554320 gb AAM63425.1 putative glycosylation enzyme [Arabidopsis thaliana] gi 21700835 gb AAM70541.1 AT5g15050/F2G14_170 [Arabidopsis thaliana] (evalue: 3.8e-24, score=112.8)	0.38	0.09
SGN-U214672	arabidopsis/peptide: At2g45220.1 68409.m05085 pectinesterase family contains Pfam profile: PF01095 pectinesterase (evalue: 7e-174, score=607.4) genbank/nr: gi 7447381 pir T10494 pectinesterase (EC 3.1.1.11) PECS-c2 - sweet orange gi 2098713 gb AAB57671.1 pectinesterase [Citrus sinensis] (evalue: 3e-176, score=620.5)	0.47	0.02
DEFENSE RESPONSE			
SGN-U220257	arabidopsis/peptide: At3g48090.1 68410.m04852 disease resistance protein (EDS1) identical to disease resistance protein/lipase homolog EDS1 GI:4454567; contains Pfam profile PF01764: Lipase (evalue: 8.7e-62, score=234.6) genbank/nr: gi 19110917 gb AAL85347.1 EDS1-like protein [Nicotiana benthamiana] (evalue: 3e-158, score=560.5)	0.39	0.1
SGN-U223757	arabidopsis/peptide: At5g23960.1 68412.m02576 terpene synthase/cyclase family non-consensus TA donor splice site at exon 4 (evalue: 6.3e-60, score=227.3) genbank/nr: gi 4105137 gb AAD02270.1 putative vetispiradiene synthase 5 [Solanum tuberosum] gi 5360687 dbj BAA82109.1 vetispiradiene synthase [Solanum tuberosum] (evalue: 5e-140, score=498.4)	0.6	0.01

... continued ...

TABLE 1 Cont.

ENERGY PATHWAYS			
SGN-U215146	arabidopsis/peptide: At1g78570.1 68408.m08402 NAD-dependent epimerase/dehydratase family similar to dTDP-glucose 4,6-dehydratase from Aneurinibacillus thermoaerophilus GI:16357461, RmlB from Leptospira borgpetersenii GI:4234803; contains Pfam profile PF01370 NAD dependent epimerase/dehydratase family (evaluate: 0, score=1177.5) genbank/nr: gi 15218420 ref NP_177978.1 NAD-dependent epimerase/dehydratase family [Arabidopsis thaliana] gi 25406555 pir C96814 hypothetical protein T30F21.10 [imported] - Arabidopsis thaliana gi 4836876 gb AAD30579.1 Similar to dTDP-D-glucose 4,6-dehydratase [Arabidopsis thaliana] gi 14596091 gb AAK68773.1 Similar to dTDP-D-glucose 4,6-dehydratase [Arabidopsis thaliana] gi 20148285 gb AAM10033.1 similar to dTDP-D-glucose 4,6-dehydratase [Arabidopsis thaliana] (evaluate: 0, score=1177.5)	0.49	0.02
SGN-U223571	arabidopsis/peptide: At1g56560.1 68408.m05963 alkaline/neutral invertase -related similar to alkaline/neutral invertase GI:9758657 from [Arabidopsis thaliana] (evaluate: 3e-119, score=424.9) genbank/nr: gi 15223561 ref NP_176049.1 alkaline/neutral invertase - related [Arabidopsis thaliana] gi 25354738 pir C96607 probable invertase F25P12.99 [imported] - Arabidopsis thaliana gi 9954756 gb AAG09107.1 Putative invertase [Arabidopsis thaliana] gi 21539565 gb AAM53335.1 putative alkaline/neutral invertase [Arabidopsis thaliana] gi 30725448 gb AAP37746.1 At1g56560 [Arabidopsis thaliana] (evaluate: 1e-117, score=424.9)	0.55	0.02
HORMONE RESPONSE			
SGN-U221653	arabidopsis/peptide: At1g74670.1 68408.m07941 GAST1-related protein similar to GAST1 protein precursor GB:P27057 [Lycopersicon esculentum] (induced by gibberellins, inhibited by ABA Plant J 1992 Mar;2(2):153-9) (evaluate: 1.2e-31, score=132.9) genbank/nr: gi 121689 sp P27057 GST1_LYCES GAST1 protein precursor gi 100217 pir S22151 gibberellin-regulated protein GAST1 - tomato gi 19247 emb CAA44807.1 gast1 [Lycopersicon esculentum] (evaluate: 2.1e-36, score=153.7)	0.49	0.03
SGN-U222410	arabidopsis/peptide: At1g66340.1 68408.m06909 ethylene-response protein, ETR1 identical to GB:P49333 from [Arabidopsis thaliana] (Science 262 (5133), 539-544 (1993)) (evaluate: 2.6e-46, score=181.8) genbank/nr: gi 4210924 gb AAD12777.1 ethylene receptor homolog [Solanum tuberosum] (evaluate: 4.8e-59, score=229.2)	0.6	0.06

... continued ...

TABLE 1 Cont.

LIPID METABOLISM				
SGN-U223765	arabidopsis/peptide: At5g08415.1 68412.m00922 lipoic acid synthase family similar to lipoic acid synthase from Arabidopsis thaliana [gi:3928758], from Mus musculus [gi:14669826] Pfam profile PF04055: radical SAM domain protein (evalue: 1.9e-69, score=258.8) genbank/nr: gi 18415808 ref NP_568196.1 lipoic acid synthase family [Arabidopsis thaliana] gi 20373023 dbj BAB91180.1 lipoic acid synthase [Arabidopsis thaliana] (evalue: 6.5e-68, score=258.8)	0.42	0.02	
SGN-U214975	arabidopsis/peptide: At5g46290.1 68412.m05136 3-oxoacyl-[acyl-carrier-protein] synthase I precursor (beta-ketoacyl-acp synthase I) (KAS I) (sp P52410) (evalue: 0, score=782.7) genbank/nr: gi 7433753 pir T10061 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41) precursor, chloroplast - castor bean gi 294668 gb AAA33873.1 beta-ketoacyl-ACP synthase (evalue: 0, score=793.5)	0.56	0.08	
METABOLISM				
SGN-U220719	arabidopsis/peptide: At3g54690.1 68410.m05597 sugar isomerase (SIS) domain-containing protein similar to SP Q47334 Polysialic acid capsule expression protein kpsF {Escherichia coli}; contains Pfam profiles PF01380: sugar isomerase (SIS) domain, PF00571: CBS domain (evalue: 2.2e-89, score=325.5) genbank/nr: gi 15232565 ref NP_191029.1 sugar isomerase (SIS) domain-containing protein [Arabidopsis thaliana] gi 11278593 pir T47628 sugar-phosphate isomerase-like protein - Arabidopsis thaliana gi 7258373 emb CAB77589.1 sugar-phosphate isomerase-like protein [Arabidopsis thaliana] (evalue: 8e-88, score=325.5)	0.55	0.06	
OTHERS				
SGN-U235658	putative membrane-associated salt-inducible protein [Arabidopsis thaliana]	0.33	0.02	
SGN-U237426	hypothetical protein DDBDRAFT_0219654 [Dictyostelium discoideum]	0.44	0.04	
SGN-U215755	arabidopsis/peptide: At1g72610.1 68408.m07704 germin-like protein (AtGER1) identical to germin-like protein subfamily 3 member 1 SP P94040; contains Pfam profile: PF01072 Germin family (evalue: 8.9e-74, score=273.5) genbank/nr: gi 18203684 sp Q9ZRA4 ABPA_PRUPE Auxin-binding protein ABP19a precursor gi 4098517 gb AAD00295.1 auxin-binding protein ABP19 [Prunus persica] (evalue: 3.5e-79, score=296.6)	0.44	0.03	

... continued ...

TABLE 1 Cont.

SGN-U215862	arabidopsis/peptide: At3g18890.1 68410.m02166 expressed protein similar to UV-B and ozone similarly regulated protein 1 UOS1 [Pisum sativum] GI:20339364 (evalue: 3e-122, score=436) genbank/nr: gi 21616072 emb CAC87810.2 Tic62 protein [Pisum sativum] (evalue: 5e-127, score=457.2)	0.53	0.07
SGN-U236129	dopamine beta-monoxygenase [Arabidopsis thaliana]	0.55	0.06
SGN-U214185	arabidopsis/peptide: At3g27090.1 68410.m03103 gda-1 - related similar to gda-1 GB:CAA74993 from [Pisum sativum] (evalue: 4e-114, score=408.7) genbank/nr: gi 2369766 emb CAA04664.1 hypothetical protein [Citrus x paradisi] (evalue: 2e-117, score=424.9)	0.57	0.09
PHOTOSYNTHESIS			
SGN-U234089	chlorophyll a-b binding protein 3C-like [Solanum tuberosum]	0.33	0.03
SGN-U213489	arabidopsis/peptide: At4g27440.1 68411.m03591 protochlorophyllide reductase B (PCR B/POR B) identical to protochlorophyllide reductase B SP:P21218 from [Arabidopsis thaliana] (evalue: 2e-174, score=609.4) genbank/nr: gi 21068893 dbj BAB93003.1 NADPH:protochlorophyllide oxidoreductase [Nicotiana tabacum] (evalue: 0, score=674.5)	0.34	0.03
SGN-U234089	chlorophyll a-b binding protein 3C-like [Solanum tuberosum]	0.38	0.06
SGN-U225521	arabidopsis/peptide: At5g38410.1 68412.m04170 ribulose biphosphate carboxylase small chain 3b precursor (RuBisCO small subunit 3b) (sp P10798) (evalue: 2.7e-74, score=275.8) genbank/nr: gi 132104 sp P07180 RBS3_LYCES Ribulose biphosphate carboxylase small chain 3A/3C, chloroplast precursor (RuBisCO small subunit 3A/3C) gi 68072 pir RKTO3C ribulose-biphosphate carboxylase (EC 4.1.1.39) small chain 3A precursor - tomato gi 19334 emb CAA29402.1 ribulose 1,5-biphosphate carboxylase/oxygenase [Lycopersicon esculentum] gi 19338 emb CAA29404.1 ribulose 1,5-biphosphate carboxylase/oxygenase [Lycopersicon esculentum] gi 170500 gb AAA34190.1 ribulose-1,5-biphosphate carboxylase/ oxygenase small subunit (evalue: 7.7e-97, score=355.9)	0.43	0.07

... continued ...

TABLE 1 Cont.

SGN-U225534	arabidopsis/peptide: At5g38410.1 68412.m04170 ribulose bisphosphate carboxylase small chain 3b precursor (RuBisCO small subunit 3b) (sp P10798) (evalue: 3.5e-60, score=144.1) genbank/nr: gi 132095 sp P07179 RBS2_LYCES Ribulose bisphosphate carboxylase small chain 2A, chloroplast precursor (RuBisCO small subunit 2A) (LESS 5) gi 68074 pir RKTOS2 ribulose-bisphosphate carboxylase (EC 4.1.1.39) small chain 2 precursor - tomato gi 170498 gb AAA34189.1 ribulose-1,5-bisphosphate carboxylase/ oxygenase small subunit (EC 4.1.1.39) gi 4456641 emb CAA29401.2 ribulose 1,5-bisphosphate carboxylase/oxygenase [Lycopersicon esculentum] (evalue: 1.2e-77, score=177.2)	0.43	0.01
SGN-U225538	arabidopsis/peptide: At5g38420.1 68412.m04171 ribulose bisphosphate carboxylase small chain 2b precursor (RuBisCO small subunit 2b) (sp P10797) (evalue: 8e-73, score=270.4) genbank/nr: gi 132079 sp P08706 RBS1_LYCES Ribulose bisphosphate carboxylase small chain 1, chloroplast precursor (RuBisCO small subunit 1) (LESS17) gi 68075 pir RKTOS1 ribulose-bisphosphate carboxylase (EC 4.1.1.39) small chain 1 precursor - tomato gi 170496 gb AAA34188.1 ribulose-1,5-bisphosphate carboxylase/ oxygenase small subunit gi 295814 emb CAA29400.1 ribulose 1,5-bisphosphate carboxylase/oxygenase [Lycopersicon esculentum] (evalue: 8.4e-95, score=348.6)	0.45	0.01
SGN-U212938	arabidopsis/peptide: At5g54270.1 68412.m06111 light-harvesting chlorophyll a/b binding protein, putative (evalue: 4e-137, score=484.6) genbank/nr: gi 115794 sp P27489 CB23_LYCES Chlorophyll A-B binding protein 13, chloroplast precursor (LHCII type III CAB-13) gi 72748 pir CDTO33 chlorophyll a/b-binding protein type III precursor (cab-13) - tomato gi 19277 emb CAA42818.1 LHCII type III [Lycopersicon esculentum] (evalue: 8e-146, score=518.5)	0.56	0.08
M14444	Tomato chlorophyll a/b-binding protein gene Cab-3C, complete cds	0.58	0.01
SGN-U213031	arabidopsis/peptide: At1g20340.1 68408.m02290 plastocyanin similar to plastocyanin GI:1865683 from [Arabidopsis thaliana] (evalue: 3.6e-49, score=191.8) genbank/nr: gi 130271 sp P17340 PLAS_LYCES Plastocyanin, chloroplast precursor gi 100238 pir S05303 plastocyanin precursor - tomato gi 19300 emb CAA32121.1 unnamed protein product [Lycopersicon esculentum] (evalue: 8.7e-60, score=232.3)	0.6	0.02
SGN-U234086	chlorophyll a/b-binding protein Cab-3C	0.6	0.01

... continued ...

TABLE 1 Cont.

PROTEIN BIOSYNTHESIS/DEGRADATION			
ID	Gene Annotation	Average of ratios	Pvalue
SGN-U237059	Eukaryotic translation initiation factor 3 subunit 10 (eIF-3 theta)	0.36	0.06
PROTEINASE INHIBITOR			
SGN-U213363	genbank/nr: gi 3913937 sp Q43710 IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi 629672 pir S43338 proteinase inhibitor - tomato gi 405582 gb AAA16881.1 proteinase inhibitor gi 462765 gb AAC37397.1 auxin-induced proteinase inhibitor gi 742004 prf 2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.43	0.03
SGN-U213363	genbank/nr: gi 3913937 sp Q43710 IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi 629672 pir S43338 proteinase inhibitor - tomato gi 405582 gb AAA16881.1 proteinase inhibitor gi 462765 gb AAC37397.1 auxin-induced proteinase inhibitor gi 742004 prf 2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.43	0.05
SGN-U213363	genbank/nr: gi 3913937 sp Q43710 IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi 629672 pir S43338 proteinase inhibitor - tomato gi 405582 gb AAA16881.1 proteinase inhibitor gi 462765 gb AAC37397.1 auxin-induced proteinase inhibitor gi 742004 prf 2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.43	0
SGN-U213363	genbank/nr: gi 3913937 sp Q43710 IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi 629672 pir S43338 proteinase inhibitor - tomato gi 405582 gb AAA16881.1 proteinase inhibitor gi 462765 gb AAC37397.1 auxin-induced proteinase inhibitor gi 742004 prf 2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.46	0
SGN-U213363	genbank/nr: gi 3913937 sp Q43710 IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi 629672 pir S43338 proteinase inhibitor - tomato gi 405582 gb AAA16881.1 proteinase inhibitor gi 462765 gb AAC37397.1 auxin-induced proteinase inhibitor gi 742004 prf 2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.47	0.09
SGN-U213363	genbank/nr: gi 3913937 sp Q43710 IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi 629672 pir S43338 proteinase inhibitor - tomato gi 405582 gb AAA16881.1 proteinase inhibitor gi 462765 gb AAC37397.1 auxin-induced proteinase inhibitor gi 742004 prf 2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.48	0.01

... continued ...

TABLE 1 Cont.

inhibitor (evalue: 3e-129, score=463)			
SGN-U213363	genbank/nr: gi 3913937 sp Q43710 IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi 629672 pir S43338 proteinase inhibitor - tomato gi 405582 gb AAA16881.1 proteinase inhibitor gi 462765 gb AAC37397.1 auxin-induced proteinase inhibitor gi 742004 prf 2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.49	0.06
SIGNAL TRANSDUCTION			
SGN-U216491	arabidopsis/peptide: At5g47530.1 68412.m05290 auxin-induced protein, putative similar to auxin-induced protein AIR12 GI:11357190 [Arabidopsis thaliana] (evalue: 1e-112, score=403.7) genbank/nr: gi 13785207 emb CAC37355.1 putative membrane protein [Solanum tuberosum] (evalue: 0, score=743)	0.54	0.04
TRANSCRIPTION FACTORS			
SGN-U220511	arabidopsis /peptide: At3g14980.1 68410.m01700 PHD finger transcription factor, putative contains Pfam profile: PF00628 PHD-finger (evalue: 1.2e-44, score=176.8) genbank/nr: gi 8777481 dbj BAA97061.1 gb AAC80581.1~gene_id:K15M2.12~similar to unknown protein [Arabidopsis thaliana] (evalue: 4.4e-43, score=176.8)	0.41	0.02
SGN-U225750	arabidopsis/peptide: At5g67420.1 68412.m07697 lateral organ boundaries (LOB) domain protein 37 (LBD37) identical to LOB DOMAIN 37 [Arabidopsis thaliana] GI:17227170 (evalue: 3.7e-54, score=208.8) genbank/nr: gi 21593577 gb AAM65544.1 unknown [Arabidopsis thaliana] (evalue: 1.1e-52, score=209.1)	0.43	0.04
SGN-U221689	arabidopsis/peptide: At5g67420.1 68412.m07697 lateral organ boundaries (LOB) domain protein 37 (LBD37) identical to LOB DOMAIN 37 [Arabidopsis thaliana] GI:17227170 (evalue: 3.7e-54, score=208.8) genbank/nr: gi 21593577 gb AAM65544.1 unknown [Arabidopsis thaliana] (evalue: 1.1e-52, score=209.1)	0.44	0
SGN-U242047	ANAC057; transcription factor [Arabidopsis thaliana]	0.54	0.04
SGN-U221678	arabidopsis/peptide: At3g54810.2 68410.m06888 GATA zinc finger protein GATA transcription factor 3, Arabidopsis thaliana, Y13650 (evalue: 1.2e-14, score=77.03) genbank/nr: gi 37572451 dbj BAC98495.1 AG-motif binding protein-5 [Nicotiana tabacum] (evalue: 3.8e-76, score=286.6)	0.55	0.03

... continued ...

TABLE 1 Cont.

SGN-U225111	arabidopsis/peptide: At4g01250.1 68411.m00154 WRKY family transcription factor contains Pfam profile: PF03106 WRKY DNA -binding domain (evalue: 2.5e-47, score=186.4) genbank/nr: gi 27817201 gb AAO23324.1 WRKY transcription factor 22 [Capsella rubella] gi 27817203 gb AAO23325.1 WRKY transcription factor 22 [Capsella rubella] (evalue: 2e-46, score=188.7)	0.56	0.02
TRANSPORT			
SGN-U236506	peptide transporter - like protein [Arabidopsis thaliana]	0.23	0.06
UNKNOWN			
SGN-U230968	No hits found	0.4	0.03
SGN-U240735	No hits found	0.43	0.09
SGN-U227318	arabidopsis/peptide: At1g52870.2 68408.m05485 expressed protein (evalue: 7e-32, score=133.7) genbank/nr: gi 30695366 ref NP_564615.3 expressed protein [Arabidopsis thaliana] gi 25405607 pir H96569 unknown protein, 54928-56750 [imported] - Arabidopsis thaliana gi 12324641 gb AAG52277.1 unknown protein; 54928-56750 [Arabidopsis thaliana] gi 14326545 gb AAK60317.1 At1g52870/F14G24_14 [Arabidopsis thaliana] gi 25090145 gb AAN72239.1 At1g52870/F14G24_14 [Arabidopsis thaliana] (evalue: 2.3e-30, score=133.7)	0.45	0.03
SGN-U218284	arabidopsis/peptide: At4g01150.1 68411.m00143 expressed protein (evalue: 9e-45, score=177.9) genbank/nr: gi 687677 gb AAB00107.1 unknown (evalue: 2.7e-43, score=178.3)	0.46	0.03
SGN-U216503	arabidopsis/peptide: At5g47920.1 68412.m05334 expressed protein similar to unknown protein (emb CAB67623.1) (evalue: 5.1e-33, score=138.7) genbank/nr: gi 15238843 ref NP_199603.1 expressed protein [Arabidopsis thaliana] gi 10177928 dbj BAB11339.1 emb CAB67623.1~gene_id:MCA23.26~similar to unknown protein [Arabidopsis thaliana] gi 26449488 dbj BAC41870.1 unknown protein [Arabidopsis thaliana] (evalue: 1.9e-31, score=138.7)	0.48	0.08
SGN-U225353	No hits found	0.54	0.01

... continued ...

TABLE 1 Cont.

SGN- U220892	arabidopsis/peptide: At1g24480.1 68408.m02787 hypothetical protein similar to EST gb AA394324 (evalue: 3.6e-88, score=322) genbank/nr: gi 25403245 pir G86378 protein F21J9.14 [imported] - Arabidopsis thaliana gi 9743354 gb AAF97978.1 F21J9.14 [Arabidopsis thaliana] (evalue: 6.2e-87, score=323.2)	0.55	0.06
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