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Research Article

Analysis of expressed sequence tags from *Citrus sinensis* L. Osbeck infected with *Xylella fastidiosa*

Alessandra A. de Souza[1], Marco A. Takita[1,2], Helvécio D. Coletta-Filho[1], Maria Luisa P.N. Targon[1], Eduardo F. Carlos[1], Eliane C. Locali-Fabris[1], Alexandre M. Amaral[1,3], Juliana Freitas-Astúa[1,4], Ana Carla O. Silva-Pinhati[1], Raquel L. Boscariol-Camargo[1], Irving J. Berger[1], Carolina M. Rodrigues[1], Marcelo S. Reis[1] and Marcos A. Machado[1]

[1]*Centro APTA Citros Sylvio Moreira, Instituto Agronômico de Campinas, Cordeirópolis, SP, Brazil.*
[2]*Centro de Pesquisa e Desenvolvimento de Recursos Genéticos Vegetais, Instituto Agronômico de Campinas, Campinas,SP, Brazil.*
[3]*Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil.*
[4]*Embrapa Mandioca e Fruticultura Tropical, Cruz das Almas, BA, Brazil.*

Abstract

In order to understand the genetic responses resulting from physiological changes that occur in plants displaying citrus variegated chlorosis (CVC) symptoms, we adopted a strategy of comparing two EST libraries from sweet orange [*Citrus sinensis* (L.) Osbeck]. One of them was prepared with plants showing typical CVC symptoms caused by *Xylella fastidiosa* and the other with non-inoculated plants. We obtained 15,944 ESTs by sequencing the two cDNA libraries. Using an *in silico* hybridization strategy, 37 genes were found to have significant variation at the transcriptional level. Within this subset, 21 were up-regulated and 16 were down-regulated in plants with CVC. The main functional categories of the down-regulated transcripts in plants with CVC were associated with metabolism, protein modification, energy and transport facilitation. The majority of the up-regulated transcripts were associated with metabolism and defense response. Some transcripts associated with adaptation to stress conditions were up-regulated in plants with CVC and could explain why plants remain alive even under severe water and nutritional stress. Others of the up-regulated transcripts are related to defense response suggesting that sweet orange plants activate their defense machinery. The genes associated with stress response might be expressed as part of a secondary response related to physiological alterations caused by the infection.

Key words: sweet orange, EST, CVC, compatible interaction.

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Introduction

The citrus industry is one of most important agribusinesses in Brazil, with highly organized segments and competitive initiatives. The Brazilian citrus industry returns an annual gross income of around 1.5 billion dollars, from exports of concentrated juice and orange sub-products such as pectin, oil, and animal food (http://www.abecitrus.com.br).

Citrus diseases can be considered the most limiting factor to increasing and sustaining productivity in Brazil. Today, the citrus variegated chlorosis (CVC), caused by *Xylella fastidiosa*, is one of the most important diseases affecting the citrus culture. Epidemiological studies demonstrated that around 40% of sweet orange groves in São Paulo State have trees with CVC (www.fundecitrus. com.br), which causes annual losses of about US$100 million to the citrus industry. The CVC symptoms include leaf mottled chlorosis, with leaves generally smaller than those from non-inoculated plants and with lower zinc and potassium levels, stunted canopy, twig die-back, and production of small fruits with a very hard rind that is normally rejected by the juice processing plants (Rossetti *et al.*, 1990; Gomes *et al.*, 2003). The symptoms occur as a result of the *X. fastidiosa's* ability to colonize the xylem vessels and disrupt the transport of water and nutrients from the root system to the canopy (Hopkins, 1995). It equally affects all sweet orange varieties and can be transmitted by leafhoppers, contaminated budwood, and seedlings (Rossetti *et al.*, 1990).

Send correspondence to Alessandra Alves de Souza. Centro APTA Citros Sylvio Moreira, Instituto Agronômico de Campinas, Rod. Anhanguera km 158, Caixa Postal 4, 13490-970 Cordeirópolis, SP, Brazil. E-mail: alessandra@centrodecitricultura.br.

Plants with CVC showed a strong correlation between disease symptoms and disorders caused by water stress, according to the hypothesis of *X. fastidiosa*-induced xylem blockage (Machado *et al.*, 1994). However, some other physiological changes are also possible. For instance, the induction of abscisic acid (ABA), commonly observed in plants with water stress as a sinalization to stress response, was not observed in plants with CVC (Gomes *et al.*, 2003), and plants colonized by *X. fastidiosa* had lower photosynthetic rates (Ribeiro *et al.*, 2003). These changes occur in plants before symptoms develop and are probably caused by low stomatal conductance, biochemical injuries to the photosynthetic machinery, and increase in alternative electron sinks (Ribeiro *et al.*, 2003).

In order to understand the genetic responses resulting from the physiological changes that occur in plants displaying CVC symptoms, we constructed two EST libraries from sweet orange [*Citrus sinensis* (L.) Osbeck] trees, with and without CVC symptoms. The CVC symptomatic plant resulted from the *X. fastidiosa* infection. Using an *in silico* hybridization strategy, we found different genes that have significant variation in their transcript frequency. Understanding the role of these transcripts could help to explain the physiological changes that occur in plants with CVC.

Materials and Methods

Preparation of cDNA libraries

The libraries were prepared from mRNA isolated from leaves of Pera sweet orange [*C. sinensis* (L.) Osbeck] grafted on Rangpur lime (*C. limonia* Osbeck) 24 months after grafting and growing under greenhouse conditions. The leaves were collected from both non-inoculated and CVC-diseased plants at the same developmental stage. The diseased plants maintained under greenhouse were obtained by graft inoculation using infected material (buds from symptomatic branches) from a commercial orchard. From 6 to 8 months after inoculation, some infected plants showed CVC symptoms and the presence of bacteria was confirmed by PCR using *X. fastidiosa* specific primers (Pooler and Hartung, 1995).

Total RNA from leaves was extracted using Trizol reagent (Invitrogen). The Poly(A+) RNA was isolated from 1 mg of total RNA with the polyATtract mRNA Isolation System (Promega Corporation, Madison, WI). The SuperScript Plasmid System with Gateway Technology for cDNA Synthesis and Cloning (Invitrogen) was used for the library construction. All the steps were done according to the manufacturers' instructions.

The sequencing reactions were performed using the Big Dye Terminator DNA sequencing Kit (Applied Biosystems). Sequencing was done in the ABI 3730 (Applied Biosystems).

In silico hybridization and functional annotation

For comparison of the libraries, we performed an *in silico* hybridization analysis. A total of 9,536 transcripts from the non-infected library and 6,408 from the infected library were analyzed. The *in silico* hybridization methodology included a clusterization of all transcripts from both libraries using the CAP3 tool (Huang and Madan, 1999) with the default parameters. Furthermore, the relative abundance of transcripts was calculated for each tentative consensus, normalizing abundances to 10,000 read libraries. The differential transcription was evaluated using statistical verification (Audic and Claverie, 1997). We considered differential expression as the possibility of a random transcript abundance distribution that for a given tentative consensus was equal to or less than 5%. Automatic categorizations over the tentative consensus were performed as well, using the Munich Center for Proteins and Sequences Functional Categories (MIPS) v. 1.3 (http://mips.gsf.de/proj/funcatDB/search_main_frame.html). Comparative genomics was carried out through tentative consensus comparison against the GenBank protein database, using the Blastall implementation of BLAST algorithm (Altschul *et al.*, 1997).

Results and Discussion

EST assembly and functional classification

We obtained 15,944 ESTs, clusterized in 4,066 contigs (consensi sequences), by sequencing two cDNAs libraries constructed from non-inoculated and CVC-diseased sweet orange leaves. Thirty-seven contigs were identified with significant variation on transcription. This could result from either an increase in transcription levels or decrease in the turnover of the mRNAs. In this subset, 21 were up-regulated and 16 were down-regulated in plants with CVC (Table 1 and 2).

To further understand the physiological changes that occur in plants with CVC, we investigated the function of the differentially expressed genes in CVC plants. The contig sequences matching previously identified plant transcripts were automatically assigned to functional classes according to MIPS v. 1.3. Some transcripts had to be manually categorized based on the best match with BlastX. The largest class of sequences with known functions was predicted to play roles in metabolism (32%). The majority of the remaining sequences were distributed among defense (13%) and protein with unknown functions (13%). The functional distribution of transcripts sequences was similar to that found in *Theobroma cacao* treated with inducers of defense response (Verica *et al.*, 2004).

Identification of down-regulated transcripts

The main functional categories of the down-regulated genes in CVC-diseased plants were also associated to metabolism, protein modification, energy and transport facili-

Table 1 - Functional categorization of the down-regulated genes in sweet orange plants exhibiting citrus variegated chlorosis symptoms

Functional category	p- value[1]	Relative abundance of reads		Match of genes up-regulated by *X. fastidiosa*	Organism	Accession number[2]
		Without Xf	With Xf			
01. Metabolism	0.0209	79.69	62.42	putative myo-inositol 1-phosphate synthase	*A. thaliana*	AAC49172
	0.0042	13.6	1.56	putative acetyltransferase	*A. thaliana*	XP_468393
	0.0000	12.58	0.00	cytochrome P450 CYP98A1	*Sorghum bicolor*	AAC39316
	0.0004	14.68	0.00	glycosylasparaginase - like protein	*A. thaliana*	CAB93711
	0.0026	17.82	3.1211	germin-like protein	*Hordeum vulgare* subsp. *vulgare*	CAC32847
	0.0101	11.53	1.56	pectinesterase	*A. thaliana*	AY143950
02. Energy	0.0003	19.92	1.56	light harvesting chlorophyll a/b-binding protein	*Nicotiana sylvestris*	BAA25390
	0.0360	61.87	51.49	Rubisco activase	*O.sativa*	BAC78572
	0.0059	9.43	0.00	photosystem I subunit O precursor	*Guillardia theta*	CAH04628
05.Protein synthesis	0.0366	30.41	20.28	ribosomal protein	*A. thaliana*	25407522
06. Protein fate (folding, modification, destination)	0.0235	9.43	1.56	FtsH metalloprotease - like protein	*O. sativa*	BAD37477
	0.0017	15.72	1.56	putative subtilisin protease	*O. sativa*	XP_481633
11. Cell rescue, defense and virulence	0.0087	14.68	3.12	putative GDSL-motif lipase/hydrolase protein	*Agave americano*	AAS75127.1
67. Transport facilitation	0.0042	13.63	1.56	aquaporin PIP1b2	*Brassica oleracea*	AAG23180
	0.0256	19.92	9.36	putative aquaporin/plasma membrane intrinsic protein	*A.thaliana*	AAN31817
99. Unknown	0.0462	53.48	46.81	putative protein	*A. thaliana*	AAO33591. 1

[1]Probability of having the putative gene differentially expressed only by chance (significant for $p < 0.05$).
[2]http://www.ncbi.nlm.nih.gov.

tation (Table 1). Among these genes, we found the heat-inducible FtsH, a pectinesterase, a germin-like protein and a hydroxylase-like cytochrome P450 (CYP98A3 family), proteins that could be directly involved in the response induced by the disease.

The heat-inducible *ftsH* gene encodes an ATP-dependent protease targeted to plastids. This protein is considered a major enzyme involved in progressive degradation (gradual degradation of oligopeptides and amino acids) (Sakamoto, 2006). A common feature of light stress in plants, algae, and cyanobacteria is the light-induced damage of the photosystem II complex (PSII). The FtsH complex alone is able to degrade damaged subunits of this complex (Nixon *et al.*, 2005). The transcripts related to photosynthesis were also down-regulated in plants with CVC. Previous research showed a decrease on net photosynthesis, transpiration, and respiration rates, stomatal conductance and leaf water potential in plants affected by CVC (Ribeiro *et al.*, 2003). According to this author, in CVC-diseased plants the damage present in symptomatic leaves (chlorosis) together with the high resistance to water flux in xylem vessels are responsible for the decrease in photosynthesis. Moreover, Queiroz-Voltan and Paradela Filho (1999) found chloroplasts totally damaged in chlorotic regions present in CVC-symptomatic leaves. In fact, stress conditions may result in reduced repair of the PSII by inhibiting either the synthesis of ATP (Allakhverdiev *et al.*, 2005) or the synthesis *de novo* of almost all of the light-inducible genes (Allakhverdiev *et al.*, 2002) which somehow might affect the expression of FtsH, an ATP-dependent metalloprotease. Therefore the down-regulation of these photosynthesis-associated genes is perhaps a consequence of disorders that occur in the photosynthetic apparatus of CVC symptomatic plants. These observations indicate the existence of a complex network of regulatory interactions and coordination of photosynthesis in response to senescence. Also, other genes encoding components of the photosynthetic machinery such as a light harvesting chlorophyll a/b-binding protein, rubisco activase and photosystem I subunit O precursor, were down-regulated in plants with CVC.

In addition, the ESTs analyses also showed one transcript involved in carbohydrate metabolism (pectinesterase). This transcript was significantly repressed under

Table 2 - Functional categorization of the up-regulated genes in sweet orange plants exhibiting citrus variegated chlorosis symptoms

Functional category	p- value[1]	Relative abundance of reads		Match of genes up-regulated by *X. fastidiosa*	Organism	Accession number[2]
		Without Xf	With Xf			
01.Metabolism	0.0000	9.43	53.05	isoflavone reductase related protein	*Populus trichocarpa*	CAA06709
	0.0002	1.04	17.16	isoflavone reductase-like protein	*Populus balsamifera* subsp. *trichocarpa*	CAA06709
	0.0000	7.34	95.19	xyloglucan endotrans-glycosylase related protein	*Medicago truncatula*	AAU89382
	0.0040	3.14	15.60	putative polygalacuronase isoenzyme 1 beta subunit	*Bruguiera gymnorrhiza*	BAB60850
	0.0002	0.00	14.04	ankyrin-like protein	*A. thaliana*	BAB02273
02. Energy	0.0225	1.04	7.80	Phosphoribulokinase, chloropl ast precursor	*O. sativa*	XP_462675
08. Cellular transport and transport mecha-nisms	0.0025	0.00	9.36	ABC1 transporter like protein	*Nicotiana plumbaginifolia*	CAC40990
10. Cellular commu-nication/signal trans-duction mechanism	0.0025	0.0	9.36	leucine-rich repeat protein putative	*Citrofortunella mitis* (calamondin)	AAP23944
11. Cell rescue, de-fense and virulence	0.0063	0.00	7.80	drought-inducible cysteine proteinase RD21A precursor	*Pisum sativum*	CAC41636
	0.0025	4.19	18.72	putative GDSL-motif lipase/hydrolase protein	*Agave americana*	AAS75127.1
	0.0002	0.00	14.04	putative lipoxygenase	*Lycopersicon esculentum*	CAA05280
	0.0327	32.50	43.69	putative peroxidase	*A. thaliana*	CAA66961
	0.0000	0.00	29.65	Cu/Zn superoxide dismutase-like protein	*Populus tremula* x *Populus tremuloides*	CAC33845
13 Regulation of / in-teraction with cellular environment	0.0388	0.00	4.68	metallothionein-like protein	*Elaeis guineensis*	CAB52585
14 Cell fate	0.0025	0.00	9.36	protein disulphide isomerase	*Zea mays*	AAX09961
25.Development (systemic)	0.0010	0.00	10.92	pollen specific protein SF21	*A. thaliana*	AAD10160
63. Protein with binding function	0.0000	4.19	63.98	lectin-related protein precursor	*Citrus x paradisi*	gb|AAG38522.1
99. Unknown	0.0002	1.04	17.16	putative protein	*A. thaliana*	AT5G26220
	0.0025	0.00	9.36	putative protein	*A. thaliana*	AT1G47330
	0.0004	0.00	12.48	putative protein	*A. thaliana*	AT1G29050
	0.0088	5.24	17.16	putative protein	*A. thaliana*	AT2G05540

[1]Probability of having the putative gene differentially expressed only by chance (significant for $p < 0.05$).
[2]http://www.ncbi.nlm.nih.gov, for the unknown proteins accession number refers to http://mips.gsf.de/cgi-bin/proj/thal/search_gene.

CVC condition. Some carbohydrates are part of the plant cell wall, making up to 90% of its composition. These carbohydrates can be divided into three groups: cellulose, hemicellulose, and pectin (Vries and Visser, 2001). This transcript might represent a gene involved in pectin metabolism. Pectin and pectin changes affect cell wall strength, porosity, ion-exchange capacity, cell adhesion, and other aspects of plant development and pathogen response (Guan and Nothnagel, 2004). Hence, a repression in carbohydrate metabolism, mainly pectin, could facilitate the bacterial colonization of the vessels.

Cytochrome P450 monooxygenases from the CYP98 family catalyze the meta-hydroxylation step in the phenylpropanoid biosynthetic pathway, and repression in such a gene may lead to reduction of plant defense and production of some phenolic compounds, modification of lignin composition and impairment in plant development (Abdulrazzak *et al.*, 2006). Hence, the down-regulation of this gene may be partially involved in the symptoms observed in plants with CVC, which include reduction in fruit size and poor development of plants.

Similarly, germin-like proteins are involved not only in stress response but also in plant development and cell wall biogenesis, C-compound and carbohydrate catabolism, osmotic regulation, photoperiodic oscillation, and defense as well (Çaliskan, 2000; Patnaik and Khurana, 2001). According to the automated categorization based on the MIPS, confirmed by a manual analysis, the germin-like protein gene that was repressed in plants with CVC is likely to be involved in C-compound carbohydrate catabolism and/or stress response, biogenesis of cellular components, and/or energy (respiration). Therefore, repression of such an important gene could possibly lead to various effects in the plant, including the reduction in growth and development observed in symptomatic plants.

In our experiment, two types of membrane transporter facilitator transcripts were down-regulated during the pathogen infection. Many aquaporins act as water channels and are thought to play an important role in plant water relations. One of the subfamilies of the aquaporins, the plasma membrane intrinsic proteins, are generally down-regulated in leaves of plants under a gradual water stress (Alexandersson *et al.*, 2005). This is probably the same situation occurring in plants with CVC.

Identification of up-regulated transcripts

The majority of the up-regulated transcripts in plants with CVC were associated to metabolism and defense response (Table 2). Among the transcripts functionally categorized as carboydrate metabolism, one contig coding was identified for a protein with xyloglucan endotransglycolase/hydrolase activity (*XTH*) (EC 2.4.1.207). The corresponding transcript was 13-fold more abundant in infected compared to non-infected leaves. These proteins are encoded by a multigene family (Xu *et al.*, 1996; Rose *et al.*, 2002) including an *Arabidopsis* touch gene named *TCH4* that encodes a xyloglucan endotransglycosylase (Xu *et al.*, 1995). Since *XTH*s are capable of modifying the plant cell wall and *TCH* genes are involved in responses to environmental stimuli, xyloglucan endotransglycosylases/hydrolases are thought to play important roles altering cell wall properties in response to environmental stresses (Braam *et al.*, 1997). Indeed, Xu *et al.* (1996) showed that expression of *TCH*4 is dramatically up-regulated in response to several environmental stimuli as well as the growth-enhancing hormones, auxin and brassinosteroids. In addition, the authors found different sensitivities of the *XTH* genes to environmental and hormonal stimuli. They concluded that differential regulation of expression of this complex gene family suggests a recruitment of related cell wall-modifying enzymes that may control the properties of cell walls and tissues during development and in response to environmental cues. Induction of an *XTH* herein presented in response to *X. fastidiosa* suggests some kind of reorganization of cell walls during the development of CVC as a consequence of the whole stress response observed. It has been postulated that *XTH*s carry out various functions, including wall loosening, wall strengthening, integrating new xyloglucans into the wall, trimming xyloglucan strands that are not tightly stuck to the surface of cellulose, fruit softening, and hydrolysing xyloglucans particularly during xylem formation. However, it is only clear that xyloglucan endotransglucosylase cuts and joins xyloglucans (reviewed by Cosgrove, 2005). The consequences of this biochemical activity for wall properties under CVC condition still needs further investigation.

Infected plants also showed induction of transcripts representing putative defense- and stress-related genes that code for protein disulfide isomerase, peroxidase, superoxide dismutase, metallothionein-like protein, dehydration-responsive protein RD22, ankyrin-like protein, lipase, lipoxygenase and isoflavone reductase-like proteins.

Cells monitor protein misfolding and trigger rapid responses to a variety of abiotic stresses (Rao and Bredesen, 2004). One of these events is the endoplasmic reticulum (ER) stress that results from the accumulation of unfolded or misfolded protein in the ER. This stress activates signal transduction pathways in an attempt to maintain osmeostasis of the ER and are known as the unfolded protein response (UPR); this results in a rapid adjustment of chaperone levels and redox activation or accumulation of stress-specific chaperones (Winter and Jakob, 2004). UPR involves the participation of protein disulfide isomerases (PDIs), which are well-known molecular chaperones (Wilkinson and Gilbert, 2004). This type of chaperones contains thioredoxin (TRX) domains that help the formation of proper disulfide bonds during protein folding (Houston *et al.*, 2005). The induction of a gene encoding a PDI in CVC diseased plants could reflect a disorder in ER homeostasis and a need for an increase in the correct folding of proteins.

Plants express a large number of isoenzymes modulated by signals related to cellular detoxification of reactive oxygen species, which may accumulate for different reasons, including wounding or pathogen attack (Dietz *et al.*, 2006). Transcripts representing genes involved with oxidative stress were up-regulated in plants with CVC, such as Peroxidases and Copper/Zinc superoxide dismutase. The function of either class of enzyme remains only speculative on citrus trees affected by *X. fastidiosa*. Peroxidases act on hydrogen peroxide (H_2O_2) and Superoxide dismutase catalyzes the conversion of superoxide radicals (O_2^-) to hydrogen peroxide and molecular oxygen (O_2). Three distinct evolutionary families of Superoxide dismutase are known, and the Copper/Zinc binding family is one of them (Lin *et al.*, 2002). In CVC affected trees, xylem vessels are partially blocked, or sectored, by bacterial biofilm (Souza *et al.*, 2005). In response, the tree may modulate the level of superoxide radicals (O_2^-), triggering Superoxide dismutase to produce hydrogen peroxide, which can further react with the phenol group of monolignol, in the presence of peroxidase, to produce lignin components. Lignin gives me-

chanical strength to plant tissues such as xylem vessels, and may also function in defense for the same reason. Lignin is formed in many plants in response to wounding. Therefore, it is possible that CVC affected plants orchestrate related genes which attempt to restrain additional infections and potential enzymatic damage caused by *X. fastidiosa* biofilm.

A gene encoding a putative metallothionein protein, up-regulated in the citrus plants infected with *X. fastidiosa,* is probably related to the homeostasis of metal ions or heavy metal binding (Cu, Fe, and Zn). According to Rauser (1999), during sap translocation from the root system to the leaves, through the xylem, citrate and histidine are the main ligands for Cu, Ni, and Zn. Possibly, the presence of these putative up-regulated genes in plants with CVC could be related to a strategy of the plant to keep the transport of these ions to supply the nutritional deficit, since the presence of the pathogen causes obstruction of the vessels and consequent reduction in nutrient transport.

There are other transcripts possibly induced by the stress condition caused by CVC, such as the dehydration-responsive protein RD22. The up-regulation of this gene could be related to a typical response to drought observed in plants with CVC. This protein was shown to be induced by ABA (Abe *et al.*, 1997). Synthesis of ABA is not a feature of plants with CVC (Gomes *et al.*, 2003) and therefore the physiological conditions are different. This was evidenced in our analysis where only this gene, related to ABA, showed differential expression. Moreover, it seems to respond to different stimuli and protect the plant in adverse conditions (Abe *et al.*, 1997). Another putative gene, possibly up-regulated due to the damage caused by water stress, was an ankyrin-like protein. This gene has high sequence similarity to a locus tagged on chromosome 3 of *Arabidopsis thaliana* (AT3G23300) with a putative methyltransferase function. The DNA methyltransferase (DNA MTase) family of enzymes catalyzes the transfer of a methyl group to DNA, which serves for a wide variety of biological functions, spanning from epigenetic events in mammals (Pradhan and Esteve, 2003) to general transcriptional repression, and consequently gene regulation, in different eukaryote organisms (Goll and Bestor, 2005). Analysis using the Conserved Domain Search (NCBI) also indicated the presence of a methyltransferase domain in the citrus ankyrin-like protein. Regarding the potential triggering events, the AT3G23300 locus codes for a dehydration-responsive protein which is similar to the early-responsive dehydration stress ERD3 protein, also from *A. thaliana*. In the case of citrus plants affected by *X. fastidiosa*, trees undergo visible stress, commonly displaying symptoms of twig die back, overall lack of vigor and changes in fruit size and quality. Thus, it is possible that the gene represented by ankyrin-like responds to *X. fastidiosa* as would happen under a dehydration condition. If that is true, maybe affected plants would then modify patterns of gene expression in several pathways using methylation process: the ankyrin-like related gene would play a role at least in some subset of responsive genes related to the symptoms seen on affected branches or in the whole tree in severe cases.

Transcripts representing genes with homology to lipoxygenase and a lipase with a GDSL motif (GDSL-lipase) were significantly up-regulated in CVC-diseased plants when compared to non-inoculated ones. However, a possible isoform of GDSL-lipase was also down-regulated in the same conditions (Table 1). The alignment of the two different GDSL-lipase using the Blast2seq tool showed that they diverge significantly in their N-terminal portion, but are rather well conserved in their C-terminal part, including the GDSL motif. The alignment also shows that they have 76% of identical residues and 86% of positive residues in the conserved region. At the nucleotide level they also show 76% of identical residues in the regions showing similarity. However, there is also a region in the middle of the sequences that does not align since it is not conserved. Therefore these two proteins are different isoforms that may have distinct functions depending on the environmental conditions to which the plant is exposed. In addition, genes with homology to lipases were found to be required for SA-dependent induction of defense responses (SAR) (Jakab *et al.*, 2003). Lipoxygenases catalyze the formation of compounds involved in plant defense responses, acting as either signaling molecules for wound-induced responsive genes or as antimicrobial substances (León *et al.*, 2002; Roopashree *et al.*, 2006).

Two transcripts representing genes that code for isoflavone reductase-like proteins were found to be significantly up-regulated under CVC condition. It has been long known that phenylpropanoids - and isoflavonoids in particular - are involved in the production of phytoalexins and, hence, antimicrobial activities (Preisig *et al.*, 1990; Nicholson and Hammerschmidt, 1992; Tahara and Ibrahim, 1995). Therefore, over-expression of genes involved in isoflavonoid biosynthesis might be expected as a response of the plant to infection.

Concluding Remarks

The presence of *X. fastidiosa* in susceptible plants leads to CVC. This disease induces water and nutritional stresses that are part of the physiological changes observed in the plants. These changes lead to modification of the gene expression pattern by either increasing or decreasing the levels of several different transcripts. Among the down-regulated genes, four were related with photosynthesis, possibly reflecting the phenotype of chlorophyll degradation observed in plants with CVC. However, sweet orange infected with *X. fastidiosa,* but displaying no CVC symptoms, showed up-regulation of several genes associated with photosynthesis, probably as a result of genetic responses of the plant to the damage caused by the bacterium

(Souza *et al.*, this issue). According to Ribeiro *et al.* (2003), sweet orange plants infected with *X. fastidiosa* showed reduction in photosynthesis before the development of symptoms.

Regardless of the damage caused by CVC, this disease does not kill affected plants. There may be adaptation mechanisms that could help to keep the plants alive. Genes related to reorganization of cell walls, ions transport and water stress were up-regulated in plants with CVC and could participate in this process.

The transcripts up-regulated in plants with CVC associated with defense response suggest that the sweet orange plants activate their defense machinery, which is not sufficient to block the disease. The whole process of pathogen recognition may be triggered later, after the bacteria are already established within the plant, a common characteristic in susceptible response to pathogen. Another possibility is that these genes are expressed as a secondary response due to the physiological effect caused by the infection, like nutritional and water stress.

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