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Identificação de genes de *Citrus sinensis* com expressão dependente da proteína PthA de *Xanthomonas citri* e isolamento de elementos *cis* regulatórios ligantes de PthA

Tese apresentada ao Instituto de Biologia para obtenção do Título de Doutor em Biologia Funcional e Molecular, na área de Bioquímica.

Orientador: Prof. Dr. Celso Eduardo Benedetti

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À minha mãe Romilda, esta pequena obra, resultado de alguns anos de trabalho longe de casa.

"Tudo que é feito com tempo o tempo respeita"
(Leonardo da Vinci)

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RESUMO

O cancro cítrico resulta da interação compatível entre a bactéria *Xanthomonas axonopodis* pv. *citri* e *Citrus* spp. A doença não tem cura, é de fácil disseminação e difícil controle. O cenário é preocupante, pois a doença diminui drasticamente o rendimento e a qualidade dos frutos de plantas infectadas, ocasionando um forte impacto econômico na citricultura mundial. Os principais sintomas do cancro cítrico, resultantes dos processos de hipertrofia (aumento do volume celular) e hiperplasia (aumento da divisão celular), são dependentes da proteína efetora PthA de *X. citri*. PthA integra a família de fatores de transcrição conhecida como efetores ativadores de transcrição (*transcription activator-like* ou TAL). O principal homólogo de PthA é o efetor AvrBs3 de *X. campestris* pv. *vesicatoria* que atua regulando a transcrição de genes do hospedeiro em benefício do patógeno. A similaridade entre estas proteínas gira em torno de 97%, sugerindo, portanto, função semelhante para PthA.

Através de uma série de microarranjos, investigou-se o perfil de expressão gênica de laranja doce (*Citrus sinensis*) dependente de PthA (*X. citri*) e de PthCs de *X. aurantifolii*, uma bactéria que causa cancro cítrico apenas no limão galego e que, em laranja doce, induz uma reação de hipersensibilidade. Desta forma, verificou-se a regulação positiva ou negativa de uma série de genes. Os PthCs regularam negativamente genes associados à sinalização por auxina e induziram a expressão de genes de defesa e silenciamento gênico. Em contrapartida, PthAs induziram uma série de genes intimamente relacionados aos sintomas de cancrose, incluindo: genes associados aos processos de aumento e divisão celular, síntese e remodelamento de parede celular, bem como genes envolvidos na sinalização por auxina e giberelina. Neste sentido, efetuou-se o isolamento de regiões promotoras de cinco genes, os quais são potencialmente regulados por PthA. A análise destas regiões revelou a presença de um possível TATA-box notavelmente semelhante àquele encontrado no gene *upa20*, denominado *UPA*-box (*up-regulated* por AvrBs3), sugerindo que estes genes poderiam ser transativados por PthA em citros. De fato, ensaios de retardamento de mobilidade eletroforética (*electrophoretic mobility shift assay* ou EMSA), demonstraram a ligação específica de PthA2 e 4 ao TATA-box encontrado na região promotora do gene que codifica

uma proteínas relacionada à patogênese (*pathogenesis-related proteins* ou PR). Este resultado corrobora com a hipótese de que os efetores TAL atuam como proteínas ligadoras de elementos TATA.

Finalmente, experimentos de co-imunoprecipitação de cromatina (ChIP) e co-transformação demonstraram, ainda que em resultados preliminares, que particularmente PthA4 é capaz de transativar *pr5 in planta*.

Embora o cancro cítrico ainda não seja completamente entendido a nível molecular, os dados aqui apresentados sugerem fortemente a ação de PthAs como fatores de transcrição, bem como aponta candidatos à regulação positiva intimamente associados aos processos de hipertrofia e hiperplasia. Além disso, as regiões promotoras aqui isoladas podem ajudar no desenvolvimento de novas estratégias para a geração de plantas resistentes à cancrose.

ABSTRACT

Citrus canker is a result of a compatible interaction between *Xanthomonas axonopodis* pv. *citri* and *Citrus* spp. There is no cure for citrus canker, and the disease is easily spread and difficult to be managed. The scenario is threatening since the disease dramatically diminishes the quality of fruits in infected plants leading to great economic losses for the world citrus producers. The main citrus canker symptoms known as hypertrophy (cell enlargement) and hyperplasia (cell division) are PthA-dependent. PthA is an effector protein from *X. citri* which belongs to the TAL effectors (*transcription activator-like*) family. The closest homologue of PthA is AvrBs3 from *Xanthomonas campestris* pv. *vesicatoria*, a TAL effector that acts as a transcriptional factor to modulate host transcription to the pathogen´s benefit. Similarity shared by these two proteins is around 97%, suggesting that PthA plays a similar role in the citrus host.

Through a number of microarray experiments, we investigate the gene transcription in sweet orange (*Citrus sinensis*) in response to the transient expression of PthA from *X. citri* or PthC from *X. aurantifolii*, pathotype C, a bacteria that causes citrus canker in Mexican lime but in orange trigger a hypersensitive response in sweet orange. We observed that PthCs down-regulated various auxin signaling genes and induced the expression of genes involved in defense and gene silencing. On the other hand, PthAs induces several genes implicated in canker development such as cell division and elongation, cell-wall synthesis and remodeling, synthesis, mobilization and signaling of auxin and gibberellin. Promoter regions of PthA-induced genes were isolated and shown to have predicted PthA and PthC binding sites at or near their putative TATA boxes. Moreover, competition gel shift assays confirmed that PthA4 shows preferential binding to the TATA box of the pathogenesis-related (*pr5*) gene promoter, supporting the idea that TAL effectors may act as general TATA-binding proteins.

Finally, both chromatin immunoprecipitation (ChIP) and co-transformation assays demonstrated however as preliminary results, that PthA4 is able to transactivate *pr5 in planta*.

Albeit the molecular mechanism by which citrus canker develop remains elusive at the molecular level, we provided data supporting the notion that PthA acts as a transcriptional factor, as well as identified PthA-induced genes associated with hypertrophy and hyperplasia. Furthermore, the promoter regions isolated here might be useful to obtain citrus plants resistant to the canker bacteria.

LISTA DE ABREVIAÇÕES

AD	Domínio ácido de ativação transcricional (*acidic transcription activator-like domains*)
CD	Dicroísmo circular (*circular dichroism*)
cDNA	DNA complementar (*complementary deoxyribonucleic acid*)
ChIP	Imunoprecipitação de cromatina (*chromatin immunoprecipitation*)
CHS	Chalcone sintase (*chalcone synthase*)
DEPC	Dietilpirocarbonato (*diethylpyrocarbonate*)
DLS	Espalhamento de luz dinâmica (*dynamic light scattering*)
DNA	Ácido desoxirribonucléico (*deoxyribonucleic acid*)
dNPTs	Desoxirribonucleotídeos trifosfatados (*deoxynucleotide triphosphates*)
DTT	Ditiotreitol (*dithiothreitol*)
EDTA	ácido etilenodiamino tetra-acético (*ethylenediamine tetraacetic acid*)
EMSA	Ensaio de retardamento de mobilidade eletroforética (*electrophoretic mobility shift assay*)
EST	Etiquetas de sequências expressas (*expressed sequence tag*)
ETI	Imunidade disparada por efetores (*effector-triggered immunity*)
ETS	Susceptibilidade disparada por efetores (*effector-triggered susceptibility*)
HR	Resposta de hipersensibilidade (*hypersensitive response*)
IPTG	Isopropil *β*-D-galactopiranosídeo (*isopropyl β-D-1-thiogalactopyranoside*)
LB	Meio de cultura de bactérias "Luria Bertani"
LPS	Lipopolissacarídeos (*lipopolysaccharides*)
MAMPs/PAMPs	Padrões moleculares associados a microrganismos/patógenos (*microbial- or pathogen-associated molecular patterns*)
MAPK	Proteínas quinases associadas à mitose (*mitogen-activated protein kinase*)
MS	Meio de cultura "Murashige & Skoong"

NB-LRR	Domínios de ligação a nucleotídeo-repetições ricas em leucina (*nucleotide binding-leucine rich repeat*)
NLS	Domínios de localização nuclear (*nuclear localization signals*)
NO	Óxido nítrico (*nitric oxide*)
OD	Densidade ótica (*optical density*)
PCR	Reação em Cadeia de Polimerase quantitavivo (*quantitative polymerase chain reaction*)
PDB	Banco de dados de estrutura de proteínas (*protein data bank*)
PMSF	Fluoreto de fenilmetalsulfonila (*phenylmethanesulphonylfluoride*)
PPR	Repetições pentatricopeptídeo (*pentatricopeptide repeat*)
Proteína Avr	Proteína de avirulência (quando em itálico refere-se ao gene)
Proteína R	Proteína de resistência (quando em itálico refere-se ao gene)
PRRs	Receptores de reconhecimento padrão (*pattern recognition receptors*)
PRs	Proteínas relacionadas à patogênese (*pathogenesis-related proteins*)
PTI	Resposta de defesa disparada por PAMPs (*PAMP-triggered immunity*)
RAV	resíduos de aminoácidos variáveis
RD2	Domínio de repetição de PthA2 (*repeat domain of PthA2*)
RLK	Receptores do tipo quinase (*receptor-like kinases*)
RMN	Ressonância Magnética Nuclear (*nuclear magnetic resonance*)
RNA	Ácido ribonucléico (*ribonucleic acid*)
ROS	Espécies reativas de oxigênio (*reactive oxygen species*)
SAXS	Espalhamento de raios-X a baixo ângulo (*small-angle X-ray scattering*)
TA	Temperatura ambiente
TAL	Efetores ativadores de transcrição (*transcription activator-like*)
TPR	Domínio típico de repetição tetratricopeptídeo (*tetratricopeptide repeat-like domain*)
TTSS	Sistema secretório tipo III (*type III secretion system*)
UPA-box	genes up-regulados por AvrBs3

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INTRODUÇÃO

O cancro cítrico: características e o impacto na citricultura brasileira

O Brasil detém posição de destaque no setor da citricultura mundial, perfazendo 40% da produção de laranja e 60% na produção do suco de laranja concentrado. O país exporta US$ 2 bilhões em suco de laranja, equivalente a 80% do mercado mundial. Apesar da importância da fruta na produção do suco, a laranja *in natura* não é tão apreciada pelos grandes centros importadores como os EUA e a Europa, em função da ocorrência de várias doenças que depreciam a qualidade do fruto, e principalmente pelo receio de introdução de novas doenças no país. Dentre elas, o cancro cítrico recebe destaque pelo impacto econômico que abrange território nacional, principalmente no Estado de São Paulo (Rodrigues e Baldini, 2002; Massari e Belasque Jr., 2006; Neves *et al.*, 2007).

Descrito pela primeira vez no Brasil na década de 50, o cancro cítrico é causado por uma infecção bacteriana que atinge folhas, ramos e frutos do hospedeiro. A bactéria responsável pelo cancro cítrico é da família *Xanthomonadaceae*, gênero *Xanthomonas*, (Bitancourt, 1957; Amaral, 2003; Schubert e Sun, 2003). Existem dois grupos filogeneticamente distintos de *Xanthomonas* que afetam plantas cítricas: o primeiro é o grupo Asiático, também conhecido como cancrose A, causado pela *Xanthomonas axonopodis* Starr & Garces emend. Vauterin, *et al.*, pv. *citri* (Hasse) Dye (Xac) [syn. *Xanthomonas campestris* pv. *citri* (Hasse) Dye] que compõe a forma mais agressiva e abrangente da doença, atacando praticamente todas as variedades de citros sendo, portanto, a de maior impacto econômico; o segundo é o grupo da América do Sul, que se subdivide em dois pelas linhagens de *X. axonopodis* pv. *aurantifolii* B e C, também conhecidas como cancrose B que infecta, preferencialmente, o limão verdadeiro (*Citrus limon*) e a cancrose C que se restringe à infecção do limão galego (*Citrus aurantifolia*) (Gabriel *et al.*, 1989; Gottwald *et al.*, 2002; Brunings e Gabriel, 2003; Schubert e Sun, 2003).

Os sintomas do cancro cítrico são oriundos dos processos de hiperplasia (intensa divisão celular) e hipertrofia (aumento do volume celular) (Duan *et al.*, 1999). O resultado é a formação de lesões circulares, semelhantes a um calo pustuloso de coloração inicial

amarela ou branca, que depois adquire um tom de marrom e textura áspera, podendo ser ou não circundados por halos cloróticos amarelos (Figura 1). Mais tarde, as lesões evoluem culminando em erupções na superfície foliar, por onde a bactéria evade o tecido da planta, facilitando sua disseminação através de agentes bióticos (insetos) e abióticos (vento e/ou chuva). Também se observa o fenômeno conhecido como anasarca, um acúmulo de água e nutrientes no apoplasto (espaço intercelular), onde a bactéria prolifera. Tecidos jovens como folhas de brotações e frutos nas primeiras fases de crescimento são mais susceptíveis (Gabriel, 2001; Brunings e Gabriel, 2003).

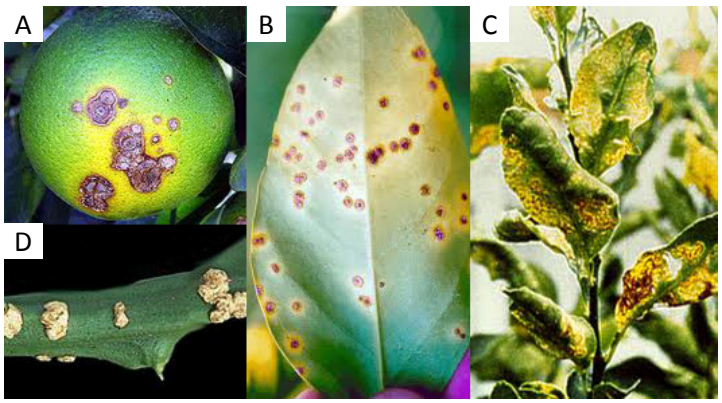

Figura 1. Sintomas característicos do cancro cítrico em estágio avançado, afetando folhas (**B** e **C**) frutos (**A**) e ramos (**D**). Fotos adaptadas de http://www.fundecitrus.com.br.

Nos frutos as lesões podem ser bastante intensas, depreciando consideravelmente sua qualidade bem como seu valor agregado. Em casos onde a infecção é severa, ocorre a queda prematura das folhas e frutos, diminuindo o rendimento das plantas de 20 a 50% (Gianessi *et al.*, 2002). Além da redução na produção, as medidas preventivas necessárias para o controle do cancro acabam por onerar o citricultor. Dentre as quais estão: a aquisição de mudas sadias; instalação de quebra ventos; descontaminação do material de colheita e veículos utilizados no pomar; pulverização do pomar com solução cúprica e a erradicação de plantas infectadas, bem como aquelas vizinhas num raio mínimo de 30 metros. Ainda, o Fundo de Defesa da Citricultura (Fundecitrus) recomenda a inspeção do pomar pelo menos três vezes ao ano e preconiza que, uma vez constatado índice maior que 0,5% de

contaminação no talhão, todas as plantas devem ser eliminadas (Gianessi *et al.*, 2002; Amaral, 2003; Koller *et al.*, 2006; Behlau *et al.*, 2007).

No entanto, recentemente foi aprovada uma nova legislação de controle do cancro cítrico que aboliu essa necessidade. A erradicação constitui a medida preventiva contra o cancro cítrico de maior importância devido a sua eficácia na contenção da doença. Desde 1999 o Fundecitrus realiza anualmente um levantamento amostral da incidência do cancro cítrico no estado de São Paulo. Em 2008 a incidência foi de apenas 0,17% dos talhões infestados, representando mais de 99,8% dos talhões diagnosticados livres da doença. No entanto, a atenuação da metodologia de erradicação, conforme previsto comprometeu o sucesso da campanha de erradicação e facilitou o ressurgimento da doença. Prova disso é o aumento da incidência da doença constatada no ultimo levantamento, que saltou de 0,14% em 2009 para 0,44% em 2010, refletindo portanto os primeiros resultados negativos financeiros e ambientais da nova legislação (Belasque Jr. *et al.*, 2009; Belasque Jr. *et al.*, 2010). Fonte para maiores detalhes: http://www.fundecitrus.com.br.

A infecção ocorre através de ferimentos ocasionados por insetos ou choques mecânicos de outra natureza e por aberturas naturais como os estômatos (células especializadas que realizam trocas gasosas), funcionando como porta de entrada para a bactéria (Brunings e Gabriel, 2003; Melotto *et al.*, 2006; Schulze-Lefert e Robatzek, 2006). Em condições ideais, onde para o desenvolvimento da doença é necessária a presença de uma lâmina de água na superfície das folhas e temperatura entre 25 e 30ºC, os sintomas iniciam-se cerca de 5 a 8 dias após a infecção (Schubert *et al.*, 2003; Schubert e Sun, 2003). Em alguns casos, a infecção é facilitada por insetos herbívoros como, por exemplo, a larva minadora dos citros (*Phyllocnistis citrella*), que também ocasiona atraso no desenvolvimento da planta. Estudos demonstraram que as galerias foliares formadas por este inseto na fase larval favorecem significativamente a susceptibilidade de infecção, uma vez que foi observada a formação de lesões típicas de cancrose seguindo os caminhos feitos pelas larvas (Figura 2). Neste sentido, foi adotado também como medida preventiva o controle químico/biológico da larva minadora (Gianessi *et al.*, 2002; Amaral, 2003; Schubert e Sun, 2003; Jesus Jr. *et al.*, 2006).

O cancro cítrico não tem cura e não se verifica resistência propriamente dita nas variedades de citros. A literatura relata para a tangerina uma "resistência moderada" à doença em condições de campo. No entanto, foi observado que não se trata de uma resistência fisiológica, visto que todas as variedades de citros desenvolvem os sintomas característicos da cancrose após a inoculação artificial do patógeno. Assim, a resistência observada em algumas cultivares de citros, como no caso da tangerina, é atribuída a fatores morfológicos como a distribuição, tamanho e numero de abertura dos estômatos permitindo a entrada da bactéria (Brunings e Gabriel, 2003). Neste sentido, é de grande importância entender como a doença se desenvolve bem como as respostas proferidas pelo hospedeiro, uma vez que a partir daí, estratégias para gerar linhagens de citros resistentes ou tolerantes ao cancro cítrico poderão ser elaboradas.

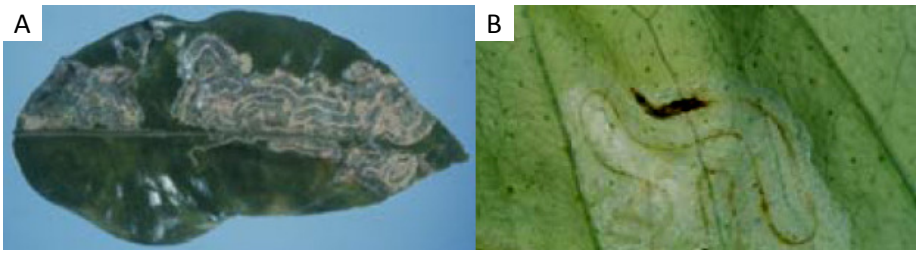

Figura 2. Sintomas do cancro cítrico associados à larva minadora do citros (*P. citrella*). Galerias causadas pela larva na parte adaxial (**A**) e abaxial (**B**) de folhas de laranja. Fotos adaptadas de Schubert *et al.* (2003).

Interação planta-patógeno

Logo após ganhar o espaço intercelular via estômatos, a bactéria busca condições favoráveis para sua proliferação com o objetivo de colonizar o hospedeiro e se multiplicar. Para tanto, os fitopatógenos possuem um arsenal de ferramentas moleculares prontas para superar ou esquivar-se dos obstáculos impostos pelo hospedeiro. Não obstante, as plantas desenvolveram estratégias notáveis de defesa que visam bloquear as tentativas de ataque impedindo o desenvolvimento da doença. Portanto, nessa "guerra" molecular travada entre patógeno e hospedeiro, perdura aquele evolutivamente melhor equipado, seja para atacar ou defender.

Sistema imune vegetal: estratégias de defesa

Rotineiramente, assim como os mamíferos as plantas lidam com agentes agressores o tempo todo, sejam eles bióticos ou abióticos. No entanto, diferentemente dos animais, as plantas são sésseis e não possuem células móveis que circulam por todo o organismo monitorando e combatendo intrusos. Por esta razão, cada célula vegetal deve ser por si só capaz de detectar, identificar e responder a um determinado patógeno (Jones e Dangl, 2006; Mehta *et al.*, 2008).

Recentemente, o que se tem observado é a existência de um sistema de defesa vegetal notavelmente complexo, que consiste no manejo dos níveis de expressão gênica adequado a situações distintas (Jones e Dangl, 2006). Em outras palavras, de acordo com a necessidade, a planta induz ou suprime a produção de determinadas moléculas por meio da alteração dos níveis de expressão de um ou mais genes. Por exemplo, numa situação de estresse osmótico, genes que codificam proteínas relacionadas à morte celular são ativados, por outro lado, quando um agressor é detectado, digamos um fungo, os genes induzidos codificam toxinas e demais componentes necessários para defesa (Dangl e Jones, 2001; Jones e Dangl, 2006; Bittel e Robatzek, 2007). Assim, parte do sucesso evolutivo das plantas deve-se a essa alta capacidade de adaptação às mais diversas situações.

Em geral, durante a interação planta-patógeno, as plantas respondem à infecção basicamente de duas maneiras: uma que consiste no reconhecimento dos padrões moleculares associados a microrganismos/patógenos (*microbial- or pathogen-associated molecular patterns* ou MAMPs/PAMPs), moléculas que são conservadas e essenciais na maioria dos microrganismos, incluindo aqueles não patogênicos, e outra que envolve uma resposta a fatores de virulência do patógeno, que pode ser direta ou indireta, que se dá através do reconhecimento desses fatores por proteínas do hospedeiro ou através da detecção de seus efeitos, respectivamente (Dangl e Jones, 2001; Jones e Dangl, 2006).

A "primeira linha" de defesa envolve o reconhecimento dos MAMPs por receptores (*pattern recognition receptors* ou PRRs) localizados na superfície da célula hospedeira. Dentre os MAMPs conhecidos, os mais bem caracterizados são: a flagelina, componente majoritário do flagelo, órgão bacteriano para mobilidade; os lipopolissacarídeos

(*lipopolysaccharides* ou LPS), um glicolipídio presente nas membranas externas de bactérias Gram-negativas e o fator de alongamento bacteriano Tu (*bacterial elongation factor Tu* ou EF-Tu), uma das proteínas mais abundantes e conservadas em bactérias (Bittel e Robatzek, 2007; He *et al.*, 2007; Van Wees *et al.*, 2008).

Gradativamente, os receptores dos MAMPs, bem como seus respectivos epítopos vem sendo identificados. O receptor para a flagelina (*flagelling sensing 2* ou FLS2) possui um domínio extracelular envolvido na interação receptor-ligante, um domínio transmembrana e um domínio intracelular quinase citoplasmático, este ultimo, responsável pela ativação da cascata de sinalização por MAPK. O epítopo ativo da flagelina (flg22), capaz de induzir resposta de defesa em plantas, compreende um peptídeo de 22 resíduos aminoácidos, referente a uma região altamente conservada da região N-terminal da flagelina. Após o reconhecimento do flg22 pelo FLS2, o complexo ligante-receptor é internalizado ativando o processo de defesa (Gómez-Gómez e Boller, 2000; Chinchilla *et al.*, 2006; Mészáros *et al.*, 2006; Robatzek *et al.*, 2006).

Por outro lado, já o receptor dos LPS é ativado por uma parte altamente conservada do Lipídio A, que é suficiente para induzir respostas de defesa em *Arabidopsis* (Kunze *et al.*, 2004). É curioso, ainda, que os LPS também pareçam estar envolvidos na sinalização de simbiose. Recentemente, um artigo relatou que o LPS de *Sinorhizobium meliloti* suprime a expressão de genes associados à defesa em culturas de células de *Medicago truncatula* (Tellström *et al.*, 2007).

Não menos importante, o receptor (EFR) do EF-Tu, identificado em *Arabidopsis* como sendo da subfamília XII de receptores do tipo quinase (*receptor-like kinases* ou RLK), é sensível a um peptídeo *N*-acetilado que compreende os primeiros 18 resíduos de aminoácidos (elf18) do EF-Tu e é, por sua vez, suficiente para ativar resposta de defesa. Ambos EFR e FLS2 integram a mesma subfamília de receptores e mediam um conjunto de respostas de defesa comum em plântulas de *Arabidopsis* tratadas independentemente com os MAMPs elf18 e flg22. Embora exista evidencias de interação entre EFR e elf18, a indicação de trafego intracelular é suportada apenas pela presença de um motivo típico de endocitose (Kunze *et al.*, 2004; Zipfel *et al.*, 2006).

Os MAMPs não são encontrados nos hospedeiros e, embora não possuam necessariamente papel na patogenicidade, fortuitamente induzem resposta de defesa no hospedeiro. De maneira geral, as respostas típicas de defesa induzidas pelos MAMPs incluem: morte celular programada; produção de espécies reativas de oxigênio (*reactive oxygen species* ou ROS); óxido nítrico (*nitric oxide* ou NO); sinalização por hormônios; espessamento da parede celular através da deposição de glicoproteínas (calose); síntese de compostos antimicrobianos e ativação da cascata de sinalização por proteínas quinases associadas à mitose (*mitogen-activated protein kinase* ou MAPK) que, por sua vez, ativam a transcrição de diversos genes de defesa (Abramovitch e Martin, 2004; Altenbach e Robatzek, 2007; Bittel e Robatzek, 2007; He *et al.*, 2007; Van Wees *et al.*, 2008). A Figura 3 ilustra resumidamente as repostas de defesa supracitadas.

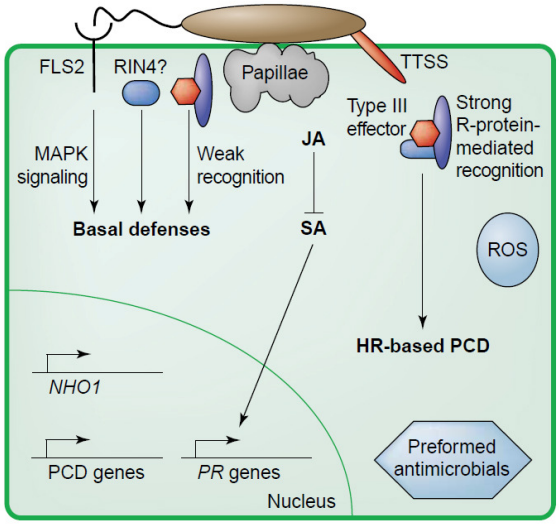

Figura 3. Esquema resumido das repostas de defesa basais induzdas por PAMPs/MAMPs. Neste caso a detecção da flagelina, reconhecida pelo receptor FLS2, induz diferentes respostas de defesa. FLS2: receptor para flagelina; RIN4: proteína guardiã de *Arabidopsis*; TTSS: sistema secretório tipo III; JA: ácido jasmônico; SA: ácido salicílico; ROS: espécies reativas de oxigênio; HR: resposta de hipersensibilidade; PCD: morte celular programada; PR: proteína relacionada à patogênese. Figura extraída de Abramovitch e Martin (2004).

Além disso, foi demonstrado que os MAMPs induzem o fechamento dos estômatos em resposta à presença de um intruso em potencial, impedindo assim o acesso do mesmo ao

mesófilo. Esse resultado atribuiu aos estômatos, até então destinados apenas para trocas gasosas, papel no sistema imune de plantas (Melotto *et al.*, 2006; Schulze-Lefert e Robatzek, 2006).

Até o momento, a comunidade científica reconhece a importância dos MAMPs no que diz respeito à indução de respostas de defesa em plantas. No entanto, também se especula a dualidade de papel, que já começa a ser observada em alguns casos, como para os LPS, que atuam também como fator importante na sinalização simbiótica (Bittel e Robatzek, 2007). É provável que essa dualidade exista para outros MAMPs conhecidos. Permanece ainda a perspectiva de que muitos outros MAMPs, bem como seus respectivos papéis sejam descobertos.

A "segunda linha" de defesa, em geral ocorre dentro da célula do hospedeiro através da expressão dos genes de resistência (*R*). O isolamento de inúmeros genes *R* demonstrou que estes codificam proteínas NB-LRR, assim denominadas em função da presença de domínios de ligação a nucleotídeo (*nucleotide binding* ou NB) e domínios ricos em leucina (*leucine rich repeat* ou LRR). Os domínios LRR participam ativamente nas interações proteína-proteína e são responsáveis pelo reconhecimento de proteínas efetoras implicando na indução de resposta de defesa (Gómez-Gómez e Boller, 2000; Chinchilla *et al.*, 2006; Chisholm *et al.*, 2006; Altenbach e Robatzek, 2007; Chinchilla *et al.*, 2007).

O modelo que descreve a interação planta-patógeno, conhecido como gene-a-gene, prevê o reconhecimento de um gene de avirulência (*avr*) do patógeno por um gene *R*, numa interação tipo receptor-ligante (Flor, 1971). Funcionalmente, o reconhecimento é resultado da interação entre o produto dos genes *avr/R* implicando numa resposta de defesa, tornando a cepa avirulenta. Comumente, o resultado dessa interação é uma resposta de hipersensibilidade (*hypersensitive response* ou HR), caracterizada por morte celular no local da infecção a fim de conter o avanço do patógeno. Neste caso, o modelo de interação é dito incompatível. Por outro lado, a interação é compatível quando a interação *avr/R* não ocorre, favorecendo o progresso da doença uma vez que o fator Avr fica livre para agir (Swarup *et al.*, 1992; Yang e Gabriel, 1995; Gabriel, 2001; Brunings e Gabriel, 2003). Em resposta a patógenos biotróficos, a HR é uma estratégia de defesa eficaz. Com a morte celular programada no local da infecção, toxinas e compostos antimicrobianos são liberados

dificultando a colonização do tecido. Contudo, a indução de HR pode não ser a melhor estratégia quando se trata de um patógeno necrotrófico. Portanto, a célula vegetal é capaz de diferenciar o estilo de vida do intruso e desferir a resposta de defesa mais adequada, demonstrando a ação de um sistema imune bastante acurado (Abramovitch e Martin, 2004).

A planta possui ainda, proteínas que funcionam como guardiãs que monitoram a presença e a integridade de outras proteínas do hospedeiro. Este modelo é denominado "hipótese de guarda" e prevê a detecção de qualquer atividade de virulência por meio de uma interação indireta entre genes *R* e *avr*. Simplificando, algumas proteínas são "guardadas", ou seja, monitoradas por outras proteínas, que ao detectarem alguma alteração ou ausência da proteína guardada, induzem resposta de defesa. Estas proteínas, em sua essência, são as proteínas guardiãs (Van der Biezen e Jones, 1998).

Este modelo foi experimentalmente verificado em *Arabidopsis*. Durante a infecção, uma protease de *Pseudomonas syringae*, AvrRpt2, cliva RIN4, uma proteína do hospedeiro que é monitorada por uma proteína R, a RPS2. Ao perceber modificações ou ausência de RIN4, RPS2 ativa uma resposta de defesa (Coaker *et al.*, 2005). Este modelo não é trivial, uma vez que uma proteína pode ser monitorada por uma ou mais proteínas R e ainda ser alvo de uma ou mais proteínas Avr. Além da RPS2, outra proteína monitora RIN4, a RPM1 (Mackey *et al.*, 2002). Contudo, RPM1 detecta mudanças em RIN4 causadas apenas pelos efetores AvrRpm1 e AvrB, que ao contrario de AvrRpt2, não cliva, mas hiperfosforilam RIN4 (Mudgett, 2005; Chisholm *et al.*, 2006). Neste caso, a reposta de defesa é indireta porem específica, já que a proteína guardiã reconhece na proteína monitorada apenas as modificações causadas por uma Avr em particular. Curiosamente, na presença de AvrRpt2, RPM1 não detecta fosforilação de RIN4 causadas por AvrRpm1 ou AvrB. Em contrapartida, ambos AvrRmp1 e AvrRpt2 inibem resposta de defesa induzida por PAMPs (Ritter e Dangl, 1996; Kim *et al.*, 2005).

Finalmente, fica claro que a evolução garantiu às plantas um sistema de defesa acurado e bastante intricado, suficiente para detectar de diversas maneiras a presença de intrusos, bem como deflagrar as diferentes estratégias de ataque proferidas pelos patógenos.

Xanthomonas: "a melhor defesa é o ataque"

O sistema imune vegetal é bastante preciso e eficiente nas respostas de defesa e superá-lo é tarefa que exige dos fitopatógenos estratégias de ataque eficientes. Neste assunto, as bactérias do gênero *Xanthomonas* são reconhecidas pelo emprego de mecanismos de ataques acurados – as diferentes espécies infectam um espectro de mais de 200 famílias de plantas, sendo mais bem estudadas aquelas que afetam culturas de interesse econômico (Tabela 1) (Boch e Bonas, 2010). Do ponto de vista microbiológico *Xanthomonas* ssp. são bactérias Gram-negativas, obrigatoriamente aeróbicas em forma de bastão, apresentam um único filamento flagelar e produzem uma pigmentação amarelada (Tang *et al.*, 1991; Yang *et al.*, 1994; Brunings e Gabriel, 2003).

Tabela 1. *Xanthomonas* que afetam culturas de interesse econômico.

Patógeno	**Cultivar**	**Referência**
X. axonopodis pv. *citri*	*Citrus* spp.	(Brunings e Gabriel 2003)
X. axonopodis pv. *aurantifolii*	*C. limon* (Limão) *C. aurantifolii* (Lima)	(Brunings e Gabriel 2003)
X. campestris pv. *vesicatoria*	*Capsicum* spp. (Pimentão) *Lycopersicon* spp. (Tomate)	(Thieme *et al.*, 2005)
X. campestris pv. *citrumelo*	*Poncirus trifoliata* (Laranja trifoliata) *C. paradisi* x *P. trifoliata* (Swigle citrumelo)	(Leite *et al.*, 1994)
X. oryzae pv. *oryzae*	*Oryza sativa* (Arroz)	(Hopkins *et al.*, 1992)
X. campestris pv. *malvacearum*	*Gossypium* spp. (Algodão)	(Gabriel *et al.*, 1986)

Após invadir o tecido vegetal, via estômatos e/ou ferimentos, em busca de nutrientes, a bactéria prolifera nos espaços intercelulares do mesófilo. Durante o crescimento *Xanthomonas* spp. produz a xantana, uma goma viscosa e higroscópica de cor amarelada. Cerca de 12 genes compõem o grupo *gum* (*gum*B a *gum*M) que é responsável pela produção da xantana e altamente conservado em *Xanthomonas* spp. (Katzen *et al.*,

1998). A xantana fornece proteção contra estresses abióticos tais como desidratação e/ou compostos tóxicos (Denny, 1995). Além disso, em *X. campestris* e *X. citri*, a xantana contribui para a formação de biofilme, o que sugere proteção contra antibióticos e respostas de defesa, além de auxiliar na colonização e desenvolvimento de sintomas (Dow *et al.*, 2003; Dunger *et al.*, 2007; Rigano *et al.*, 2007; Kim *et al.*, 2009). Curiosamente, a hidratação da xantana promove o inchamento celular que auxilia na ruptura da epiderme (Tang *et al.*, 1991). Nos estágios mais avançados da infecção a ruptura da epiderme favorece a liberação da bactéria na superfície foliar que, fica então, disponível para disseminação (Yang *et al.*, 1994; Duan *et al.*, 1999).

No entanto, para completar o ciclo de vida a bactéria precisa empregar uma série de estratégias de ataque que, sinergicamente, supere as respostas de defesa e contribua para o desenvolvimento da doença. Para isso, *Xanthomonas* possui seis tipos de sistemas secretórios (de I a VI), dentre os quais, o sistema secretório tipo III (***t****ype* ***III*** ***s****ecretion* ***s****ystem* ou TTSS) recebe destaque. O TTSS é comparado a uma "seringa" molecular cujo papel é injetar um coquetel de proteínas efetoras para dentro da célula do hospedeiro. Em sua essência o TTSS consiste numa maquinaria protéica que é codificada pelo *regulon* de hipersensibilidade e patogenicidade (*hypersensitivity response and pathogenicity* ou *hrp*) (Hueck, 1998; Rossier *et al.*, 1999; Büttner e Bonas, 2002; Szurek *et al.*, 2002; Ausubel, 2005; Kay *et al.*, 2007; Veenendaal *et al.*, 2007). O funcionamento deste regulon se dá através de duas 'chaves' moleculares, o *hrpG* e *hrpX*, que numa ação coordenada regulam outros seis genes *hrp*, *hrpA-hrpF*. Estes, por sua vez garantem a formação correta do Hrp pilus que se insere na parede celular, funcionando como um duto que permite a passagem das proteínas efetoras (Wengelnik e Bonas, 1996; Wengelnik *et al.*, 1996; Huguet e Bonas, 1997).

Uma vez no interior da célula vegetal as proteínas efetoras interferem em passos importantes do metabolismo do hospedeiro em beneficio do patógeno. Essas proteínas possuem as mais diversas funções: de proteases a fatores de transcrição. Em princípio, elas funcionam como fatores de virulência, porém quando são reconhecidas ou tem seus efeitos detectados pelo hospedeiro, atuam como fatores de avirulência induzindo HR. Por esta razão, originalmente as proteínas efetoras foram definidas como proteínas de avirulência

(Avr) (Brunings e Gabriel, 2003; Schornack *et al.*, 2006). Os efetores induzem doença alterando não apenas processos metabólicos do hospedeiro necessários para o desenvolvimento da doença, mas também combatem as respostas de defesa através da supressão de eventos como: HR; calose; produção de NO; ROS; sinalização por hormônios; expressão de genes de defesa e demais processos (veja Figura 3) (Abramovitch e Martin, 2004; Nomura *et al.*, 2006; Bittel e Robatzek, 2007).

O modelo '*zigzag*' resume muito bem a atuação dos efetores durante a infecção (Figura 4). Em síntese o modelo descreve a amplitude de defesa nas diferentes condições, representada por quatro fases. Inicialmente, na fase 1 ocorre uma resposta de defesa induzida por PAMPs (*PAMP-triggered immunity* ou PTI) onde logo, na fase 2, um dado efetor trata de frustrar a PTI levando o hospedeiro a susceptibilidade (*effector-triggered susceptibility* ou ETS). No entanto, após o reconhecimento de tal efetor, na fase 3, o sistema imune é novamente ativado, onde o sistema imune é induzido pelo efetor (*effector-triggered immunity* ou ETI). Em contra partida, em casos onde a doença se instala, a seleção natural levou os efetores a evitar a ETI através da supressão de defesa, restabelecendo a ETS, caracterizando a fase 4 (Jones e Dangl, 2006). Fica claro, portanto, o importante papel das proteínas efetoras no processo evolutivo que confere a *Xanthomonas* a capacidade de causar doença.

Efetores: a família AvrBs3/PthA

Dentre as proteínas efetoras de *Xanthomonas* spp. mais estudadas destacam-se os membros da família AvrBs3/PthA, basicamente fatores de transcrição, recentemente chamados de efetores ativadores de transcrição (*transcription activator-like* ou TAL). Estruturalmente, as características essenciais para a atividade dos efetores TAL são: um domínio central composto por 34/35 resíduos de aminoácidos que se repetem em *tandem* em número variável; domínios de localização nuclear (*nuclear localization signals* ou NLS) e de ativação transcricional (*acidic transcription activator-like domains* ou AD). Essas características são comuns em fatores de transcrição de eucariotos, indicando que ao longo da evolução, os efetores TAL adaptaram-se para mimetizar esses fatores de transcrição,

com o objetivo de reprogramar os níveis transcricionais do hospedeiro em benefício do patógeno (Szurek *et al.*, 2001; Marois *et al.*, 2002; Szurek *et al.*, 2002; Kay e Bonas, 2009; Römer *et al.*, 2009b).

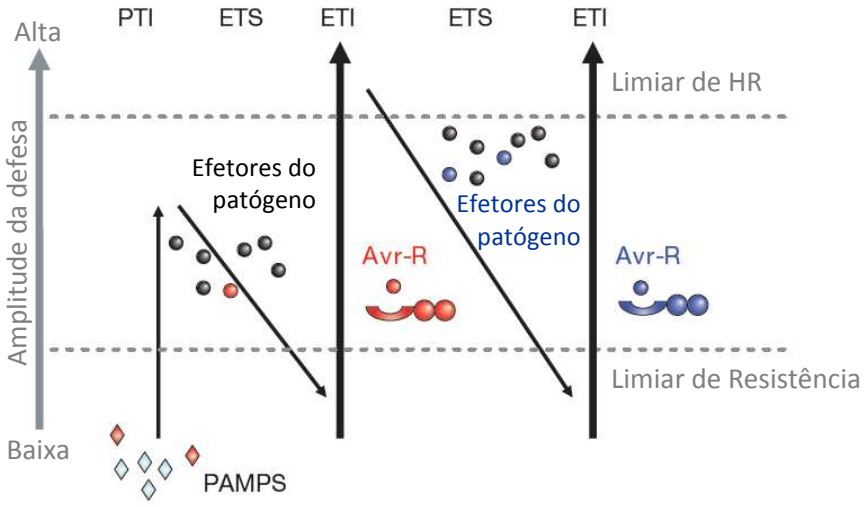

Figura 4. Modelo '*zigzag*' descreve a amplitude das respostas de defesa de plantas nas diferentes fases. PTI: resposta de defesa induzda por PAMPs; ETS susceptibilidade induzda por efetores; ETI: imunidade induzda por efetores; Avr: proteína de avirulência; R: proteína de resistência; PAMPS: padrões moleculares associados a patógenos. Figura extraída de Jones e Dangl (2006).

Atualmente, o efetor AvrBs3 de *Xanthomonas campestris* pv. *vesicatoria*, membro da família AvrBs3/PthA é o efetor TAL mais estudado. O AvrBs3 é necessário em *X. vesicatoria* para causar doença em cultivares de tomate e pimentão susceptíveis (Bonas *et al.*, 1989). Em síntese, o AvrBs3 é endereçado ao núcleo após o reconhecimento da região do NLS por uma α-importina, que juntamente com a β-importina auxilia a passagem do complexo pelo poro nuclear (Van den Ackerveken *et al.*, 1996; Szurek *et al.*, 2001; Szurek *et al.*, 2002). Uma vez no núcleo, o domínio central é importante para dimerização além de conferir atividade de ligação ao DNA. No hospedeiro susceptível o gene diretamente modulado por AvrBs3 é o *upa20*, um regulador majoritário de hipertrofia, processo biológico que resulta nos sintomas causados por *X. vesicatoria* (Herbers *et al.*, 1992; Marois *et al.*, 2002; Kay *et al.*, 2007).

Por outro lado, em *X. citri* a proteína PthA é o principal fator de patogenicidade, integra a família AvrBs3/PthA e é necessário para desencadear hipertrofia e hiperplasia em *Citrus* spp., processos biológicos que levam à cancrose (Duan *et al.*, 1999). A similaridade entre AvrBs3 e PthA é superior a 96% (Schornack *et al.*, 2006), no entanto *X. citri* possui quatro variantes de PthA, as quais se distinguem pelo número de repetições do domínio interno (Figura 5) (da Silva *et al.*, 2002; Brunings e Gabriel, 2003). A proteína PthA4 é considerada a variante essencial para induzir hipertrofia e hiperplasia e portanto, o homólogo equivalente ao AvrBs3 em *X. citri* (Al-Saadi *et al.*, 2007). Em geral, ensaios de duplo-híbrido demonstraram que as proteínas PthA interagem com diversas proteínas de laranja doce, dentre elas proteínas envolvidas no transporte nuclear como α-importina, enovelamento de proteínas e ubiquitinação associada ao reparo de DNA (Domingues *et al.*, 2010). PthA4 interage particularmente com proteínas associadas à regulação transcricional (Soprano e Benedetti, dados não publicados).

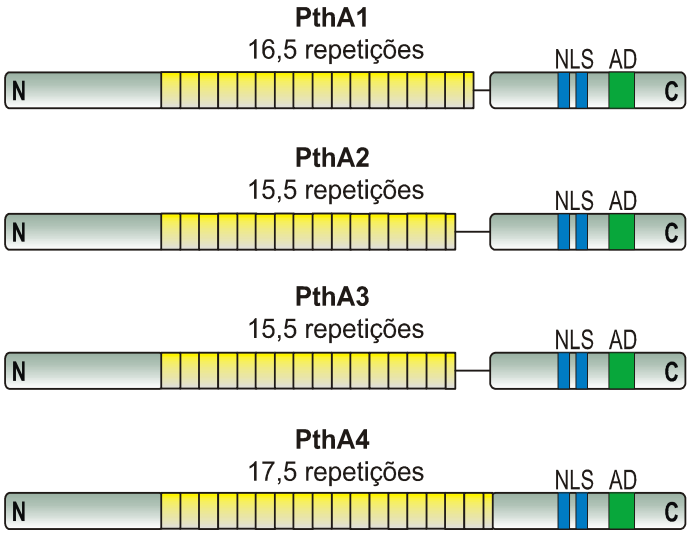

Figura 5. Características estruturais de PthA de *X. citri*. Os PthA se diferem basicamente pelo número de repetições que são encontradas no domínio interno (amarelo). Em azul estão os domínios de localização nuclear e em verde está o domínio de ativação transcricional. N: N-terminal e C: C-terminal, NLS: domínios de localização nuclear, AD: domínio ácido de ativação transcricional.

Alguns PthA também interagem com proteínas relacionadas ao metabolismo de auxina (Souza e Benedetti, dados não publicados). Curiosamente, inibidores de auxina e giberelina infiltrados em folhas de laranja atenuam os sintomas da doença, sugerindo, portanto, um papel fundamental destes hormônios no processo de desenvolvimento do cancro cítrico (Cernadas e Benedetti, 2009). Além disso, a alteração dos níveis de expressão de genes relacionados a estes hormônios não é novidade, sobretudo aqueles relacionados à mobilização e sinalização de auxina (Cernadas *et al.*, 2008). Não somente auxina, mas também a giberelina, são hormônios conhecidos por participarem no crescimento e desenvolvimento vegetal (Hutchison *et al.*, 1999; Kotake *et al.*, 2000; Sánchez *et al.*, 2004).

Neste sentido acredita-se que PthA, assim como AvrBs3, atuem como fatores de transcrição modulando o transcriptoma do hospedeiro direta ou indiretamente (através da interação com fatores de transcrição ou repressores do hospedeiro), contribuindo para o desenvolvimento da cancrose. De fato, vários genes apresentam nível de expressão elevado quando folhas de laranja doce foram infectadas com *X. citri*, sobretudo aqueles relacionados com os principais sintomas da doença (Cernadas *et al.*, 2008). Embora esses dados não sejam suficientes para apontar PthA como pivô dessa modulação, experimentos prévios demonstraram que a expressão transiente de PthA em citros é suficiente para induzir hiperplasia e hipertrofia (Duan *et al.*, 1999). Consistente com estes dados, as análises de um peptídeo de PthA contendo 1,5 repetição por Ressonância Magnética Nuclear, juntamente com estudos espectroscópicos do domínio internos de PthA2, demonstram uma estrutura secundária em α-hélice muito bem definida, que na presença de DNA sofre mudanças conformacionais que sugerem interação com DNA (Murakami *et al.*, 2010).

Recentemente, o mecanismo molecular pelo qual os efetores TAL ligam DNA foi desvendado. Em geral, os efetores TAL possuem resíduos de aminoácidos variáveis (RAV) nas posições 12 e 13 dentro de cada repetição do domínio central. O código para o reconhecimento específico de uma sequência de nucleotídeos reside nesses resíduos, onde cada RAV liga um único nucleotídeo. Assim o número e a organização das repetições determinam quantos e quais serão os nucleotídeos ligados. Portanto, é possível predizer

quais serão os alvos de ligação dos efetores TAL através da análise dos RAV (Boch *et al.*, 2009; Moscou e Bogdanove, 2009). Contudo, o mecanismo pelo qual *X. citri* provoca o cancro cítrico permanece obscuro, sobretudo o papel molecular de PthA, já que o código TAL ainda não foi experimentalmente comprovado para PthA.

Em *X. aurantifolii*, os efetores equivalentes a PthA são os PthC1 (17.5 unidades de repetição) e 2 (14.5 unidades de repetição). Muito pouco se sabe sobre os mecanismo utilizados por *X. aurantifolii* para causar doença em limão. No entanto, é interessante observar que enquanto em limão causa cancro, em laranja *X. aurantifolii* induz uma série de genes relacionada à resposta de defesa (Cernadas *et al.*, 2008). Esse resultado sugere uma resposta do tipo ETI em laranja em função da presença de PthC, portanto, em laranja estes efetores funcionariam como proteína Avr. Neste sentido, para identificar genes de resistência, seria necessário investigar o perfil de expressão gênica induzido por PthC em laranja doce.

OBJETIVOS E JUSTIFICATIVA

Embora muito tenha sido feito para o controle do cancro cítrico, do ponto de vista biológico o desenvolvimento da doença ainda não é compreendido. Considerando que proteínas PthA são essenciais para o processo de patogênese e desenvolvimento do cancro cítrico pela modulação da transcrição de genes específicos do hospedeiro, esse trabalho teve como objetivo identificar genes em citros potencialmente transativados por PthA que estejam associados ao desenvolvimento dos sintomas da doença. Além disso, através do isolamento de dois novos efetores tipo TAL de *X. aurantifolii*, PthC1 e PthC2, investigou-se o papel desses efetores na resposta de defesa observada em laranja doce. Ainda, esse trabalho objetivou o isolamento de promotores de genes de citros cuja expressão é induzida por PthA e a identificação de sítios de reconhecimento de PthA.

RESULTADOS

CAPÍTULO I: Análise do perfil de expressão gênica de citros (*Citrus sinensis*) e identificação de genes potencialmente regulados por PthA.

Introdução

Neste capítulo são relatados os experimentos realizados para investigar o perfil de expressão gênica de citros visando a busca por genes potencialmente regulados por PthA. O presente trabalho será apresentado em forma de artigo científico como submetido no jornal *Molecular Plant Pathology* (Pereira *et al.*, 2011). Como material suplementar, a Tabela S1 agrupa todos os dados referentes às análises de diferentes tratamentos realizados em experimentos de microarranjos. Em princípio, os experimentos podem ser divididos em três etapas, que envolvem os experimentos de microarranjos, isolamento e análise das regiões promotoras de genes de laranja doce e estudos de interação proteína-DNA.

Os dados de microarranjos contribuíram para identificar genes com expressão elevada em diferentes condições, com o objetivo principal de encontrar genes associados à formação de cancro cítrico ou mesmo a respostas de defesa, dependentes de PthA. Vários genes foram selecionados a partir desses resultados. Em seguida, a amplificação de regiões promotoras de genes potencialmente necessários para desencadear sintomas de cancrose resultou na identificação de elementos de regulação gênica TATA-*box*. Considerando a importância desse elemento para a regulação de genes em hospedeiros mediado por uma proteína efetora similar a PthA, experimentos de interação proteína-DNA foram realizados para investigar a afinidade de PthA por sequências promotoras de citros.

Por fim, é importante destacar que durante o andamento do presente trabalho, o genoma completo de *Citrus clementina* e *C. sinensis* foi concluído, possibilitando a posteriori a busca por regiões promotoras com sítios alvos de PthA.

ARTIGO CIENTÍFICO SUBMETIDO:

TAL effectors of *Xanthomonas citri* and *Xanthomonas aurantifolii* target sweet orange genes involved in canker development and disease resistance, respectively

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TAL effectors of *Xanthomonas citri* and *Xanthomonas aurantifolii* target sweet orange genes involved in canker development and disease resistance, respectively

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SUMMARY

Transcriptional activator-like (TAL) effectors of the AvrBs3/PthA protein family are known for their ability to selectively bind and transactivate host target genes. Although numerous targets of *Xanthomonas* TAL effectors have been reported recently, very little is still known about citrus genes specifically activated by the *Xanthomonas citri* effector proteins PthAs, required for citrus canker development. In addition, much less is known about the function or targets of the PthC effectors of *X. aurantifolii*, a *X. citri*-related pathogen that triggers a defense response in sweet oranges. Through a combination of bioinformatics, microarray analyses and protein-DNA binding assays, we have identified a number of sweet orange genes as targets of PthA and PthC proteins. Most of the genes that were up-regulated by PthAs are associated with canker development, whereas those identified as targets of PthCs are related to disease resistance. In particular, while PthAs 2 and 4 positively modulated the auxin response required for canker development, PthCs 1 and 2 down-regulated the auxin signaling and induced the expression of genes involved in defense and gene silencing. Promoter regions of PthA-induced genes were isolated and shown to have predicted PthA and PthC binding sites at or near their putative TATA boxes. Moreover, competition gel shift assays confirmed that PthA4 shows preferential binding to the TATA box of the pathogenesis-related (*pr5*) gene promoter, supporting the idea that TAL effectors may act as general TATA-binding proteins.

INTRODUCTION

Transcriptional activator-like (TAL) effectors have long been recognized as key bacterial determinants conferring both pathogenicity and avirulence in numerous plant species (Al-Saadi et al., 2007; Bonas et al., 1989; Duan et al., 1999; Shiotani et al., 2007; Sugio et al., 2007, Yang et al., 2006). However, only recently the biological function of TAL effectors as plant transcriptional regulators has been established (Boch and Bonas, 2010; Bogdanove et al., 2010; Kay et al., 2007; Römer et al., 2007; Yuan et al., 2011).

TAL effectors are unusual in the sense that they are structurally and functionally distinct from other bacterial proteins that are targeted by the type-III secretion system to the interior of the host cells (Boch and Bonas, 2010). In particular, TAL effectors have a polymorphic DNA-binding domain made of tandem repeats of 33-34 amino acids that confer DNA specificity (Boch et al., 2009; Moscou and Bogdanove, 2009). The selectivity of TAL effectors to specific DNA sequences is greatly determined by an amino acid polymorphism that occurs at positions 12 and 13 of each repeat unit. These variable residues, referred to as the repeat-variable diresidues (RVDs), are found in all TAL effectors and each specifies the preferential binding to certain DNA bases (Boch et al., 2009; Boch and Bonas, 2010; Moscou and Bogdanove, 2009). Thus, the consecutive order of RVDs within the repeat region of a TAL effector defines the DNA target sequence that the effector will recognize in host promoters. Such target sequences have been referred to as the UPT (up-regulated by TAL) boxes (Römer et al., 2009). Now that the TAL effector code has been deciphered and proved functional, it has been used to predict the UPT boxes of many naturally-occurring effectors and to generate artificial TAL effectors with new specificities, opening up new biotechnological perspectives (Li et al., 2011; Miller et al., 2011; Zhang et al., 2011).

Although several targets of *Xanthomonas* TAL effectors have been reported, very little is still known about citrus genes directly modulated by the *Xanthomonas citri* effector proteins PthAs, required for citrus canker development (Al-Saadi et al., 2007; Duan et al., 1999; Kanamori and Tsuyumu, 1998; Swarup et al., 1991). In addition, much less is known about the function or direct targets of the as yet uncharacterized PthC effectors from *Xanthomonas aurantifolii* pathotype C, a *X. citri*-related pathogen that triggers a hypersensitive response in sweet oranges (Cernadas et al., 2008). Thus, to gain insights into

the specificity and determine the biological function of distinct *X. citri* and *X. aurantifolii* effectors in more detail we used a combination of bioinformatics, microarray analyses and *in vitro* binding assays to identify sweet orange genes as potential direct targets of the PthA and PthC proteins. Here, we show that while PthAs up-regulate a great number of citrus genes associated with cell growth and expansion, PthCs on the other hand, appear to induce gene silencing and to trigger a defense response in sweet orange through the activation of particular transcriptional regulators. Contrary to PthAs, however, PthCs negatively regulated the response to auxin, which is required for canker development (Cernadas and Benedetti, 2009). Furthermore, we found that promoters of PthA-induced genes have predicted PthA binding sites that overlap with or are located adjacently to TATA box-like sequences that are similar to DNA sequences recognized by other *Xanthomonas* TAL effectors. The observation that PthAs with relatively distinct internal repeat regions have preferential binding to such sequences supports the notion that TAL effectors may in fact function as general TATA-binding proteins.

RESULTS AND DISCUSSION

Identification of citrus genes as targets of *X. citri* TAL effectors

Large-scale transcriptional analysis of sweet orange leaves challenged with *X. citri* revealed numerous genes associated with canker development, however, identification of direct targets of *Xanthomonas* TAL effectors were not possible (Cernadas et al. 2008). Here, to identify such targets we first analyzed the transcriptional changes in sweet orange leaves infiltrated with *X. citri* in the presence of cycloheximide (Ch), an approach that has been successfully used to identify targets of AvrBs3 within 9 h of bacterial infiltration (Marois, Van den Ackerveken et al. 2002).

Table 1 shows the main citrus genes up-regulated by *X. citri* which were not affected by the Ch treatment 8 h post infection (hpi). The majority of these genes are involved in ethylene and gibberellin (GA) synthesis and signaling, cell growth and defense. In particular, the genes involved in ethylene signaling encode proteins similar to AP2 ethylene-response factors (ERFs) which play roles in fruit softening and regulation of cell-wall remodeling enzymes (Tacken et al., 2010; Yin et al., 2010), whereas those involved in defense include some pathogenesis-related (PR) proteins, chitinases (CHT) and WRKY factors previously shown to be up-regulated by *X. citri* (Cernadas et al., 2008).

Next, we transiently expressed the *X. citri* PthA2 and 4 variants in sweet orange epicotyls (Figure 1) and analyzed the changes in transcription in respect to controls (epicotyls transformed with GUS). PthAs 2 and 4 were chosen because they form heterodimers and interact with a number of citrus nuclear proteins implicated in transcriptional control (Domingues et al., 2010). In addition, since PthA2 and 4 have a similar RVD composition (Figure 2A), we anticipated that they might target the same genes in citrus plants. Consistent with this idea, we observed that the transient expression of PthAs 2 and 4 in sweet orange epicotyls resulted in the up-regulation of a similar set of genes (Table 2 and Supplemental Table 1). Most notably, the genes that were up-regulated by PthAs 2 and 4 are involved in auxin, ethylene and GA signaling, cell division, cell-wall remodeling, and defense (Table 2). Many of them were previously shown to be induced by *X. citri* infection and are thought to contribute to canker development (Cernadas et al.,

2008). Some of the PthA-induced genes including PR1, PR4, PR5, CHT1, CHT3 and ACC synthase, were also up-regulated by *X. citri* in the presence of Ch (Table 1), suggesting that they may represent direct targets of PthAs. Furthermore, we noticed that genes associated with cell division like kinesin, histone and ribosomal protein genes and the homolog of the *defective embryo and meristems* (*dem*), required for cell division in meristems (Keddie et al., 1998) were preferentially up-regulated by PthA4. Two homologs of the auxin influx carrier protein AUX1 (CV706455, CX053885), required for lateral root, nodule development and morphogenesis (Bennett et al., 1996; de Billy et al., 2001; Vandenbussche et al., 2010), and two GA-regulated proteins (CX637545, CF508354) related to the cell and organ shape regulators (Kotilainen et al., 1999; Zhang et al., 2009) were also preferentially induced by PthA4 (Table 2 and Supplemental Table 1). On the other hand, genes encoding cell-wall remodeling enzymes and the homolog of the *up-regulated by AvrBs3-15* (*upa15*) were up-regulated by both PthAs (Table 2), indicating that PthAs 2 and 4 play a synergistic role as transcriptional activators in citrus cells. This idea is consistent with the fact that homologs of PthA4 having 17.5 repeat units are the only PthA variants shown to determine pathogenicity on citrus (Al-Saadi et al., 2007).

Promoters of PthA-induced genes have UPT-like sequences

The promoter regions of four *C. sinensis* genes that were up-regulated by PthAs or induced by *X. citri* independent of protein synthesis were isolated. These promoters correspond to the genes encoding the PR1 and PR5 proteins, CHIT1 and a WRKY protein (Figure 2B). The isolated sequences, which extend up to 1 kb upstream of the initial ATG contain putative TATA box-like elements within 35 to 170 bp upstream of the initial start codons (Figure 2B). At least for *pr5*, promoter-GUS fusion assays showed that this sequence drives the expression of GUS in transiently transformed citrus epicotyls, indicating that it behaves as a citrus regulatory region (Figure 2C).

To find possible PthA-binding sites in these promoters, a search matrix based on the TAL-code frequencies (Boch et al., 2009; Boch and Bonas, 2010; Moscou and Bogdanove, 2009) was created. The matrix was used since in these promoter sequences we found no

perfect matches for the predicted PthA-binding sites reported previously, which take into account only the highest frequent DNA base for the respective PthA RVDs (Boch et al., 2009) (Figure 2A). By contrast, the matrix found putative PthA-binding sites located near or at the predicted TATA-box elements in the four citrus promoters. Notably, the *pr5* promoter appears to have PthAs 2, 3 and 4-binding sites which overlap with or are adjacent to the TATA box of this promoter (Figure 2B). Similarly, a binding site for PthA4 and PthC1, a new TAL effector from *X. aurantifolii* (see description bellow) was found within the predicted TATA-box elements of the *wrky* and *pr1* promoters, respectively (Figure 2B). In addition, a possible PthA4-binding site carrying one violation of the code also overlaps with the putative TATA element of the citrus *chit1* promoter (Figure 2B). Considering that TAL effectors have been suggested to play a role as TATA-binding proteins (Römer et al., 2009; Antony et al., 2010), we compared the putative PthA and PthC binding sites encompassing the TATA box region of the citrus promoters. Surprisingly, we found that these promoter regions have a TA- followed by a C-rich stretch (Figure 2D) that remarkably resembles the consensus sequence of promoters up-regulated by AvrBs3 (Kay et al., 2009). Interestingly, the recently reported TAL effector (TALE13) from another strain of *X. citri* (Miller et al., 2011) has an RVD content that specifies the binding to TATAAATACCTTCT, which also resembles the consensus sequence shown in Figure 4D.

In silico prediction of UPTs for PthAs in *Citrus clementine* promoters

As the first fully annotated genome sequence of a citrus plant (*Citrus clementine)* became recently available (www.citrusgenomedb.org), we used the search matrix to find putative PthA-binding sites in the promoter regions of *C. clementine* genes. On average, nearly a thousand PthA-binding sites were identified. Interestingly, we found putative PthA2 and 4-binding sites in the promoters regions of the corresponding PthA-induced genes involved in cell growth, cell-wall remodeling and defense (Table 2). Gene ontology (GO) analysis also revealed, for instance, an enrichment of PthA2-binding sites in the *C. clementine* promoters of cell-wall remodeling genes, suggesting that these genes may in fact represent direct targets of PthAs 2 and 4 in citrus plants.

PthA4 preferentially binds to the putative TATA box of the *pr5* promoter

To test whether PthAs 2 and 4 recognize the putative binding sites identified within the citrus *pr5* promoter (Figure 2B), three DNA probes designated PR5-box, containing the predicted TATA-box element with the overlapping PthAs 3 and 4 sites, PR5-up, containing a PthA2 site, and PR5-down, with no PthA sites, were used in gel-shift assays (Figure 3A). In addition, to address if the DNA-binding activity of PthAs resides on its internal DNA-binding domain, as expected, a PthA4ΔID derivative lacking its repeat units was used as negative control. As shown in Figure 3B, PthA4 binds to the PR5-box at a much lower protein/DNA ratio compared to PthA2, whereas its ΔID derivative shows a weaker binding even at the highest protein/DNA ratio tested.

To test the specificity of the DNA-binding activities of PthAs 2 and 4 we performed competition gel-shift assays using the PR5-up and PR5-down probes as competitors. Figure 3C shows that while the PR5-up probe competes with PR5-box better than PR5-down and PR5-box itself, only PR5-box was able to compete with itself when PthA4 was used in the assay. These results indicated that while PthA4 prefers to bind to the TATA box-like sequence, PthA2 has preferential binding to PR5-up which carries a PthA2 site immediately upstream of the TATA-box element (Figure 3A and C).

Next, we performed competition EMSA using the predicted PthA-binding sites described previously which consider only the best frequent DNA base for the RVDs (Kay et al., 2009). As shown in Figure 3D, none of the sequences corresponding to the predicted PthAs 2 and 4 binding sites was able to compete with the PR5-box probe in gel-shift assays. However, to know whether the length of the competitor probes or adjacent sequences flanking the predicted TATA-box element were influencing the protein-DNA interactions, we replaced the TA- and C-rich sequence of the PR5-box with the previously suggested PthAs 2 and 4 DNA targets (Kay et al., 2009), as competitors 2 and 4, respectively (Figure 3E). We found that although competitor 2 competed with the PR5-box, competitor 4 did not seem to compete as much as the PR5-box itself (Figure 3F), suggesting that PthA4 has a preference for the TA- and C-rich sequence of the TATA-box element of the citrus *pr5* promoter. Moreover, our data suggest that the DNA flanking the TAL effector binding site is import to stabilize the protein-DNA interaction.

Identification of two novel TAL effectors from *Xanthomonas aurantifolii*

We showed previously that *X. aurantifolii* pathotype C, which triggers a hypersensitive reaction on sweet oranges, up-regulates numerous genes involved in defense responses including those related to cell wall reinforcement and basal resistance (Cernadas et al., 2008). To know whether the resistance response induced by *X. aurantifolii* on sweet oranges is largely or partially mediated by PthA-related effectors, we first isolated TAL effectors from the *X. aurantifolii* strain ICMP 8354. The only two effectors identified, designated PthC1 and 2, differ essentially in the number of their internal repeats (Figure 2A). PthC1 has 17.5 internal repeat units (the equivalent of PthA4), whereas PthC2 has 14.5 repeats (Figure 1). Despite the similarities shared with PthAs 2 and 4 with respect to the RVD composition, PthC1 and 2 are more related to each other and to PthB and PthC variants from other *Xanthomonas* strains (Al-Saad et al., 2007; El Yacoubi et al., 2007) than to *X. citri* PthAs. Interestingly, PthC2 is similar to a *X. citri* TAL responsible for host-specific suppression of virulence (Shiotani et al., 2007). These observations led us to test whether PthC1 and 2 would influence transcription in sweet orange associated with the hypersensitive reaction trigged by *X. aurantifolii*.

TAL effectors from *X. aurantifolii* down-regulate auxin response genes and induce defense-related genes

One of the changes in transcription upon PthC1 and 2 expression was observed in the auxin synthesis and signaling (Table 3). Curiously, all the citrus genes associated with the auxin response were down-regulated by the PthCs. Since auxin is required for canker development (Cernadas and Benedetti 2009), it seems that PthCs are contributing negatively to canker elicitation, as opposed to PthAs. However, some of the PthC-repressed genes (CV713157, CV704184, CK701644, CN182471) encode proteins homologous to Aux/IAA and bZip factors that function as negative regulators of the auxin and GA signaling (Nishimura et al., 2002; Wang et al., 2005; Weller et al., 2009; Zhang et al.,

2007). In addition, down-regulation of GH3-like genes and homologs of the indole-3-acetic acid amido synthase also indicates that PthCs would increase the active pools of auxin. Thus, it remains to be elucidated whether the general down-regulation of the auxin signaling genes observed here contribute to symptom development or not.

We also highlight the up-regulation of the citrus *upa22* homolog by PthC1 and 2 (Table 3). The fact that homologs of *upa22* and *upa15* were up-regulated by PthA and PthC proteins indicates that similar genes in different hosts can be targeted by TAL effectors with apparently "unrelated" RVD signatures. It is interesting to note that Bs3 is regarded as a deletion derivative of the YUCCA protein involved in auxin biosynthesis (Römer et al., 2009). Since AvrBs3 also targets other auxin-regulated genes (Marois et al., 2002), it is possible that Bs3 might have originally contributed for disease symptoms rather than defense. The observation that PthA and PthC proteins modulate various auxin-regulated genes indicates that distinct *Xanthomonas* pathogens found alternative ways to target the response to auxin, which has been shown to increase disease susceptibility in various plant-pathogen interactions (Domingo et al., 2009; Navarro et al., 2006; Wang et al., 2007).

Although the PthC proteins modulated a much smaller number of citrus genes compared to PthA2 and 4, they up-regulated some homologs of NAC and ERF factors involved in non-host resistance (Liu et al., 2011; Oh et al., 2005; Selth et al., 2005; Zhang et al., 2004; Zhou et al., 1997) (Table 3 and Supplemental Table 1). Surprisingly, one of the few genes that were specifically up-regulated in response to PthC2 expression encodes a homolog of the S-adenosyl-L-homocysteine hydrolase (Table 3). In Arabidopsis, S-adenosyl-L-homocysteine hydrolase, which is encoded by the *homology-dependent gene silencing* (*hog1*) gene, is required for DNA methylation-dependent gene silencing (Rocha et al., 2005). Because mutations in *hog1* resulted in genome-wide demethylation and increased transcription in Arabidopsis (Rocha et al., 2005), it is possible that induction of citrus *hog1* by PthC2 increase DNA methylation leading to general gene silencing. Interestingly, genome searches in *C. clementine* promoters not only revealed the presence of PthC-binding sites in various promoters of PthC-modulated genes but also found a PthC2-binding site in the promoter of the corresponding *C. clementine hog1* gene (Table 3). Thus,

expression of PthC proteins in sweet oranges under the control of the *pr5* promoter may be an alternative to generate resistance against *X. citri* strains.

PthAs and PthCs as TATA-binding proteins

The observation that AvrBs3 binds to TATA-like sequences and change the transcriptional start sites in promoters of Upa genes led the proposition that AvrBs3 may function as a TATA-binding protein (Römer et al., 2009). In addition, as observed for PthAs and PthCs, TAL effectors from *X. oryzae* have either a TATA-like element within their target sequences or they bind at or adjacently to TATA boxes of rice promoters (Antony et al., 2010). Thus, although no consensus sequence appears to exist for UPT boxes of effectors carrying distinct RVDs signatures (Römer et al., 2009), it is still possible that TAL effectors may have conserved their functionality as TATA-binding proteins by targeting host sequences that are critical for transcriptional regulation in the host.

EXPERIMENTAL PROCEDURES

Bacterial strains, plasmids and growth conditions

PthAs 2 and 4 were amplified from the *X. citri* strain 306 (da Silva et al., 2002) and cloned into pET28a and pBI121 for bacteria and plant expression, as previously described (Dominges et al., 2010). Plasmids were introduced into *Escherichia coli* and/or *Agrobacterium tumefaciens* strain EHA105 by electroporation. *E. coli* cells were cultivated at 37°C in Luria-Bertani (LB) medium, whereas *X. citri* and *A. tumefaciens* were grown in LB without NaCl at 28°C and in YEP at 30°C, respectively. Bacterial cultures were grown at different time periods until they reached the desired optical densities. Antibiotics were added to the media in the following final concentrations: ampicillin, 100 µg/ml; kanamycin, 50 µg/ml; rifampicin, 50 µg/ml; streptomycin, 25 µg/ml.

Isolation of TAL effectors from *X. aurantifolii*

PthA-related genes were amplified from total DNA extracted from the *X. aurantifolii* pathotype C strain ICMP 8435 (Cernadas et al., 2008) by PCR, using primers derived from the four *X. citri pthA* genes, and clone into the NdeI/EcoRI sites of pET28a vector. More than twenty independent clones were sequenced and only two new variants of PthA-related effectors designated PthC1 and PthC2 were identified. The sequences of these effectors were deposited in the GeneBank as ADI48327 and ADI48328 accessions, respectively.

Expression of *Xanthomonas* TAL effectors in citrus epicotyls

Agrobacterium cells transformed with pBI121 (expressing the *uid* gene) or pBI121 carrying each of the *Xanthomonas* TAL effectors in place of the *uid* gene, pBI121-*pthA2*, pBI121-*pthA4*, pBI121-*pthC1* or pBI121-*pthC2*, were used to transform sweet orange epicotyls. Epicotyls from young plantlets of *Citrus sinensis* 'Hamlin' were wounded, transversely sectioned and incubated at room temperature for 15 minutes in a fresh suspension of *A. tumefaciens* containing 100 µM acetosyringone at an optical density of 0.6 at 600 nm. Co-cultivation were performed on solidified 1x Murashige and Skoog medium supplemented with 25 g sucrose per liter, vitamin cocktail (10 $mg.l^{-1}$ thiamine-HCl, 10 $mg.l^{-1}$ pyridoxine, 1 $mg.l^{-1}$ nicotinic acid, 0.4 $mg.l^{-1}$ glycine), 100 mg of *myo*-inositol per liter and 0.2 mg of

2,4-dichlorophenoxyacetic acid per liter (pH 5.8) for 72 h in the dark (de Oliveira et al., 2009). Transformation efficiency was confirmed by western blot prior to RNA isolation and microarray analysis, as described below.

PthA expression and Western blot analysis

PthAs 2 and 4, and the truncated version of PthA4, containing only its internal domain (PthA4ΔID), were expressed in BL21(DE3) cells as 6xHis-fusion proteins and purified by affinity chromatography as previously described (Domingues et al., 2010). PthAs and PthCs expressed in citrus epicotyls were extracted with the SDS-PAGE sample buffer and resolved on a 10% SDS-polyacrylamide gel and probed with the PthA2 antiserum (Figure 1).

Plant material, bacterial infiltration

Six-month-old plants of sweet orange were obtained from certified nurseries and kept in a growth room at 25–28 °C with a 14 h photoperiod. *X. citri* cells were recovered by centrifugation and resuspended in sterile water at an optical density of 0.6 at 600 nm. Leaves were infiltrated with bacterial suspensions in water or 50µM Ch. Water and Ch only were independently infiltrated as mock controls.

RNA isolation and microarray analysis

Messenger RNA was extracted from infiltrated leaves 8 h post-inoculation as described previously (Cernadas et al., 2008). For microarray hybridization, approximately 0.6 µg of mRNA was used for the synthesis of cDNAs and biotin-labeled complementary RNAs (cRNAs) using the One-Cycle target labeling assay (Affymetrix). The processed cRNAs were used to hybridize the GeneChip citrus genome arrays (Affymetrix) according to the Affymetrix instructions. The hybridized arrays were rinsed, stained and the GeneChip images were acquired on the Affymetrix Scanner 3000–7G. Two CEL files of each treatment corresponding to two biological replicates were analyzed by the ArrayAssit software package (ArrayAssit x.5, Stratagene, USA) using the MAS5 algorithm.

Genomic DNA library construction, promoter amplification and analysis

Genomic DNA from *C. sinensis* was digested with *SphI* and a pool of fragments ranging from 500 to 2000 bp was gel-purified and ligated into the *SphI* adaptor (5'- TAATACGACTCACTATAGGGATCTGCACCAGAATTCCATG - 3') containing the site for amplification with nested oligo A (5'- GATCTGCACCAGAATTCCATG - 3'). Two reverse primers designed to amplify the 5´ coding sequences of the genes of interest were combined with oligo A in nested PCR reactions. PCR products were cloned into the pGEM-T Easy vector (Promega) and sequenced. The sequences of the sweet orange promoters were analyzed using the PLANTCARE software (Lescot, Déhais et al. 2002).

The DNA fragment extending ~500 bp upstream of the initial ATG codon of the citrus *pr5* gene was subcloned into the HindIII/BamHI sites of the pBI121 vector to drive the expression of the reporter gene *uid* (GUS). The *A. tumefaciens* strain EHA105 carrying the construct was used to transform citrus epicotyls and transient expression of GUS was detected by colorimetric assays at different time intervals after bacterial transformation (Jefferson et al., 1987).

***In silico* prediction of TAL effector binding sites**

A position specific weight matrix based on the TAL code frequencies (Boch et al., 2009; Boch and Bonas, 2010; Moscou and Bogdanove, 2009) was created and used to obtain a similarity score to promoter sequences of citrus genes. The search was performed using the MOODS algorithm (Korhonen et al., 2009), which provides a list of possible binding sites and score their similarity to the matched promoter sequences. The p-value cutoff was set to 0.003 and binding sites with scores below 3.0 were discarded. The matrix was used to find PthA and PthC-binding sites in the promoters of the sweet orange *pr1*, *pr5*, *chit1* and *wrky* genes. In addition, the matrix was used to find PthA and PthC-binding sites in promoter regions (1.5 Kb upstream of each gene) extracted from the annotated *C. clementine* genome (http://www.citrusgenome.ucr.edu/) using the homemade perl script.

Electrophoretic mobility shift assay (EMSA)

EMSA was performed using purified PthA proteins and ^{32}P-labbeled double-strand DNA probes. Single-strand complementary oligonucleotides were mixed together at the same molar ratio in the annealing buffer (10 mM Tris, pH 7.5–8.0, 50 mM NaCl, 1 mM EDTA) and incubated in a thermal cycler at 95ºC, then ramping down to 25ºC, with a 5ºC decrease in every two minutes. The oligos were labeled with deoxyadenosine 5-triphosphate [alpha-^{32}P] using the Klenow fragment (Fermentas). The binding reaction performed in 12 mM Tris-HCl, pH 8.0, 60 mM KCl, 1 mM DTT, 2.5% Glycerol, 5 mM $MgCl_2$, 0.05% NP-40, 0.2 mM EDTA contained approximately 0.5 pmol of labeled DNA and up to 0.6 µg of purified 6x His-tagged PthAs. For competition EMSA assays, the above mix was complemented with molar excesses of unlabeled non-specific or specific DNA as competitors. The binding reactions were incubated on ice for 20 min. Gel electrophoresis was performed on a 6% native polyacrylamide gel in 0.5 x Tris-borate-EDTA buffer and shift bands were detected by autoradiography.

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FIGURE LEGENDS

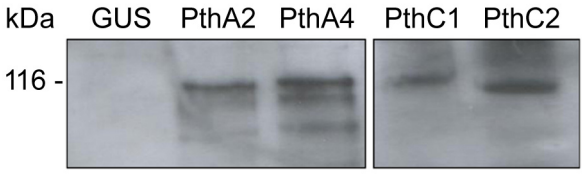
Figure 1. Transient expression of PthA and PthC proteins in citrus cells. Proteins were transiently expressed in sweet orange epicotyls after *Agrobacterium*-mediated transformation. A β-glucuronidase (GUS) construct was used as control. PthA and PthC proteins (~116 kDa) were separated on 10% SDS-polyacrylamide gels and detected by the anti-PthA2 serum.

Figure 2. Promoters of PthA-induced genes have UPT-like sequences. (A) Sequence of RVDs in PthA2, PthA4, PthC1 and PthC2 and their respective DNA bases define by the TAL code showing the first and second most frequent base only. (B) Promoter regions of four citrus genes that were up-regulated by PthAs or induced by *X. citri* independent of protein synthesis. The predicted TATA-box elements are in bold whereas the initial ATG is in bold-italic. TAL-binding sites predicted by the matrix are indicated. (C) GUS activity driven by the *pr5* promoter in transiently transformed citrus epicotyls. (D) DNA sequence alignment of the promoter regions of PthA-induced genes encompassing the putative PthA and PthC-binding sites with the predicted TATA-box elements. The consensus sequence which is rich in TA followed by a C stretch is shown and a conserved TATA element is indicated by the asterisks.

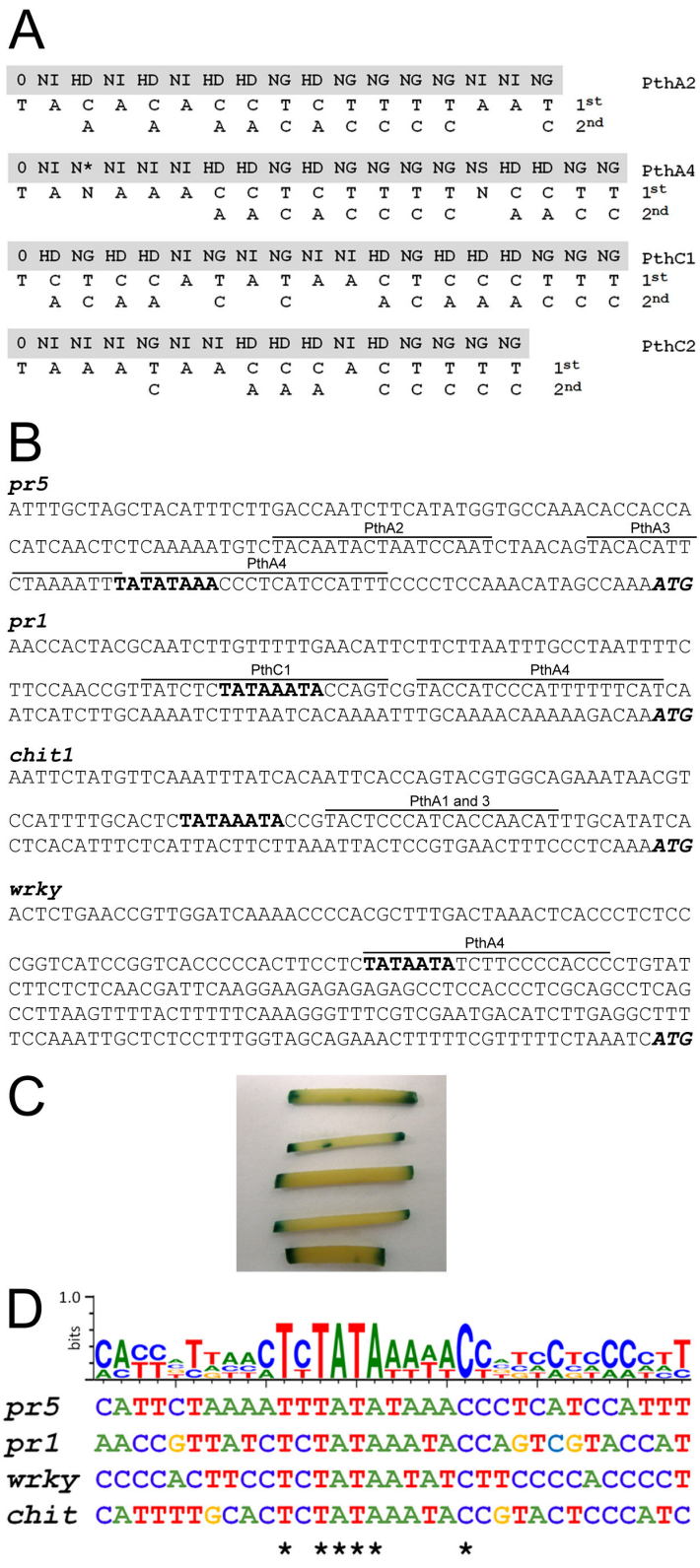
Figure 3. **PthA4 shows specificity to the TATA-box element of the *pr5* promoter**. (A) Schematic representation of the citrus *pr5* gene and its regulatory region. Three probes designated PR5-up, PR5-box and PR5-down were designed for the EMSA. The start codon ATG in PR5-down and the predicted TATA-box element in PR5-box are in bold. Negative numbers indicate the nucleotide distance from the translational start site (+1) of the *pr5* coding sequence (cds). (B) EMSA using increasing amounts of the purified PthAs incubated with the PR5-box probe labeled with [α^{32}P]-ATP. The uppermost signals correspond to the gel slots. Strong signals were particularly detected when PthA4 was incubated with the PR5-box probe (upper arrow), however, a weak DNA-binding activity by the PthA4ΔID protein was also detected at high protein concentrations (lower arrow). (C) EMSA of purified PthAs 2 or 4 incubated with the [α^{32}P]-labeled PR5-box in the

presence of increasing amounts of the unlabeled competitors PR5-up, PR5-box or PR5-down. Shifted bands are indicated by the arrows, the free probe by an asterisk whereas the probe in the absence of any competitor by "+". PthA4 appears to bind more strongly to PR5-box whereas PthA2 to the PR5-up probe. (D) Competition EMSA using the purified PthAs 2 and 4 with the [α^{32}P]-labeled PR5-box in the presence of competitors 2 (comp2) or 4 (comp4). The Comp2 (5'-ACACACCTCTTTTAAT-3') and 4 (ACAAACCTCTTTTACCTT) sequences were expected to be recognized by PthA2 and 4 (Boch et al., 2009), respectively. (E) Designed probes containing the expected PthA2 (PR5-comp2) or PthA4 (PR5-comp4) binding sites in place of the predicted TATA-box element of the citrus *pr5* promoter. (F) Competition EMSA using the purified PthAs 2 and 4 with the [α^{32}P]-labeled PR5-box probe in the presence of unlabeled PR5-comp2 or PR5-comp4 as competitors. Shifted bands are indicated by arrows. Asterisks represent the free probe, whereas "+" indicates probe without any competitor.

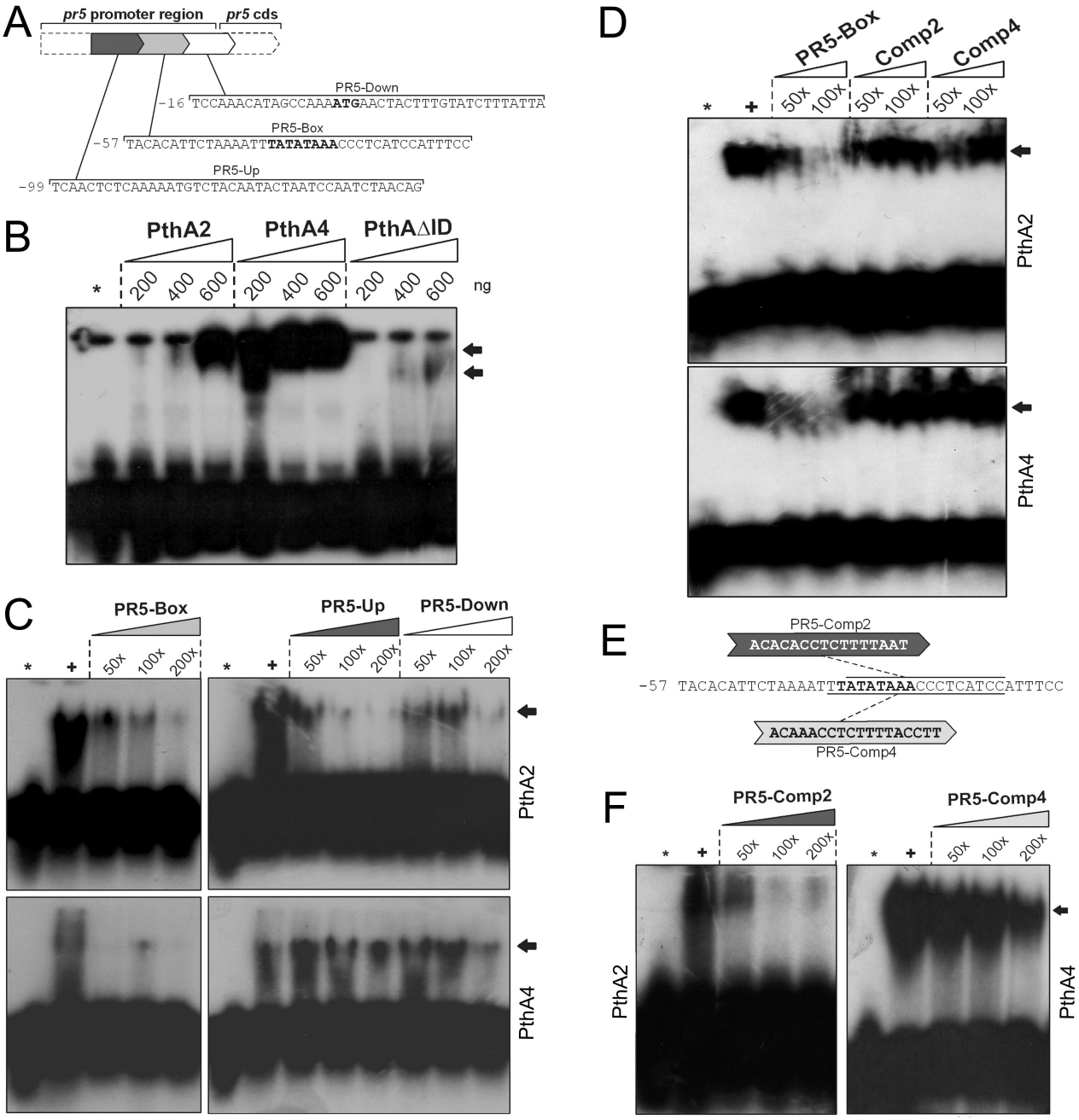
Pereira, Fig. 1



Pereira, Fig. 2



Pereira, Fig. 3



Pereira, Tab. 1

Table 1. Main citrus genes modulated by *X. citri* in the presence and absence of cycloheximide (Ch), relative controls (leaves infiltrated with water). Down-regulation is indicated by "-".

Target Description	**Citrus**	**Target**	**Fold change**		
Hormone signaling	**EST**	**Gene ID**	**Ch**	***X. citri***	***X. citri*-Ch**
ACC synthase	CX643923	CAB60722	2,8	5,3	26,7
AP2 domain transcription factor-like	BAB08875	CX299615	1,1	3,2	12,3
Ethylene response factor	BAA07323	DN617716	1,4	4,3	3,1
Ethylene response factor	NP_182011	CK937360	1,1	3,1	14,1
Geranyl geranyl pyrophosphate synthase	CX669501	BAA78047	-1,0	11,3	4,8
Homogentisate geranylgeranyl transferase	CX665915	AAV74623	1,6	8,3	15,4
Homogentisate geranylgeranyl transferase	CX301885	AAV74623	1,3	30,4	55,6
S-adenosyl-L-methionine:benzoic acid	CX669533	AAO45013	1,4	4,0	8,1
Cell division and morphogenesis					
Lateral organ boundaries (LOB) protein	BQ623314	NP_172268	1,1	3,4	8,8
High mobility group (HMG) protein	CX664460	NP_974413	1,0	3,6	4,9
Defense-regulated genes					
Pathogenesis-related protein PR1	CF653559	AAK30143	1,3	28,1	26,5
Pathogenesis-related protein PR4	CX637285	CAA41437	1,5	4,3	15,6
Pathogenesis-related protein PR4	CF835337	CAA41437	1,6	4,2	10,6
Pathogenesis-related protein PR5	CX292655	AAM21199	1,2	7,6	23,4
Pathogenesis-related protein PR5	CF836158	AAM21199	1,8	21,2	24,8
Chitinase CHI1	CX299099	AAC35981	-1,8	11,1	4,2
Chitinase CHI1	CX663308	AAC35981	-1,1	14,8	6,1
Chitinase	CX671223	CAA09110	-1,4	4,4	3,7
Acidic chitinase III	CX043703	CAA77656	1,2	5,9	12,8
Chitinase II	CF838393	S72528	1,3	5,2	15,4
WRKY-type DNA binding protein	CF828414	BAC23031	4,9	6,0	40,4
WRKY transcription factor 30	CX044130	AAR92477	1,8	3,0	9,0
WRKY transcription factor 40	CX044789	AAU04404	1,7	4,6	8,7
WRKY-type DNA binding protein	CX050828	AAP12887	2,0	4,4	20,8
WRKY family transcription factor	CN188753	NP_564792	2,4	3,8	30,5
Resistance protein RPP8-like protein	CX669576	AAP82824	2,8	4,7	15,3
Putative receptor protein kinase	CV707423	AAO42089	2,1	5,0	31,8
Cyclophilin (CsCYP)	CX299605	ACX37092	14,6	31,8	41,1
BON1-associated protein (BAP1)	CX077288	NP_182100	-1,1	9,9	4,9

Pereira, Tab. 2

Table 2. Main *Citrus sinensis* genes up-regulated by PthA2 and 4 with fold changes > 3, relative to controls (epicotyls expressing GUS), identified by microarray analysis. Non-detected genes are indicated by "nd". Predicted PthA2 and 4-binding sites found in the respective *Citrus clementine* promoters are indicated by "+".

Target Description	**PthA sites**		**Citrus**	**Target**	**Fold change**	
Auxin, ethylene and GA signaling	**PthA2**	**PthA4**	**EST**	**Gene ID**	**PthA2**	**PthA4**
AUX1-like permease			CV706455	CAC12996	nd	4,7
Auxin influx carrier protein			CX053885	AAM55306	nd	4,0
Auxin-binding protein ABP19a precursor			CK937473	Q9ZRA4	3,2	5,2
Auxin-regulated protein-like			CX675673	XP_483243	nd	5,9
Auxin-responsive GH3 family protein			CN184032	NP_194456	3,2	nd
ADR11-2 protein - soybean		+	AU300809	S33621	8,3	7,2
ACC oxidase			CX305211	AAG49361	nd	11,2
ACC synthase	+		CX643923	CAB60722	3,3	8,0
Ethylene-forming-enzyme-like dioxygenase			CX298890	AAB88878	nd	4,1
Gibberellin-regulated ribosomal protein			CX663607	CAA46273	nd	3,1
Gibberellin-regulated protein GASA5 precursor		+	CX637545	AAA98520	5,3	nd
GEG protein	+	+	CF508354	CAB45241	5,5	11,8
Cell division and expansion						
Alpha-tubulin 4		+	CV719766	AAQ92663	nd	5,3
Beta-tubulin 1		+	CX672740	AAL92118	nd	3,5
Kinesin related protein		+	CX053924	BAA01972	nd	4,3
kinesin-like protein		+	CF828325	BAB40710	nd	3,3
Dem protein		+	DN618785	T07737	nd	3,9
Histone H2A		+	CX667228	AAF65769	nd	9,7
Histone H3	+	+	CK932935	NP_910496	3,2	nd
Beta-1,3-glucanase	+	+	CV886686	CAA03908	3,8	23,2
Beta-1,3-glucanase		+	CD575247	BAA89481	nd	4,6
Beta-xylosidase			CX077158	BAB11424	8,9	14,5
Acidic cellulase			CF831790	AAB65155	81,5	42,4
Basic cellulase			CX663293	T07885	6,8	6,9
Cellulose synthase			CB293314	AAB63624	nd	5,4
Endopolygalacturonase	+	+	CX294670	AAP21999	35,4	11,7
Endopolygalacturonase	+	+	CB250319	AAC64184	26,7	nd
Polygalacturonase-like protein	+	+	CX666732	AAP33475	6,6	nd
Polygalacturonase-like protein	+		CB250305	NP_191544	23,3	nd
Arabinogalactan protein	+	+	CV709336	AAO92753	4,4	nd
Immuno-reactant natriuretic peptide			CV885460	AAM18791	3,1	10,4
Alpha-expansin 3			CF837795	AAR09170	3,1	3,7
Expansin			CV710432	AAK48848	nd	4,2
Extensin-like cell wall protein			CX672178	AAA79364	4,0	6,9
Proline-rich protein NtEIG-C29	+	+	CN182741	BAB16431	5,5	nd
Proline-rich protein	+		CX071344	AAC17605	7,0	nd
Glycosyl transferase protein – UPA15		+	CV709535	NP_19766	13,9	12,5
Pectin methylesterase isoform alpha		+	CF833607	AAF35897	9,4	5,8

Xyloglucan endotransglycosylase			CN182557	AAB39950	10,9	4,5
Defense response / secondary metabolism						
Caffeic acid O-methyltransferase II			CX045519	AAL91506	nd	8,4
Cinnamoyl CoA reductase		+	CB291269	AAP46143	nd	3,8
Acidic chitinase III			CX043703	CAA77656	nd	8,3
Basic helix-loop-helix family protein			CK937073	NP_193829	nd	7,8
Chitinase CHI1			CX292066	AAC35981	4,7	10,4
Cprd2		+	CX292181	BAB33033	nd	4,5
Disease resistance-responsive protein-related			CK934775	NP_176113	3,3	9,2
Glutathione S-transferase			DR403286	AAG30140	3,1	6,2
Monooxygenase family protein			CX302100	NP_196694	nd	5,6
NAM (no apical meristem)-like protein			DN619712	AAB81668	5,1	13,4
Pathogenesis-related group 5 protein		+	CX676279	AAB95118	3,8	6,7
Pathogenesis-related protein 4A		+	CF835337	CAA41437	nd	7,0
Pathogenesis-related protein 5-1	+ *	+	CX292655	AAM21199	5,7	13,6
Pathogenesis-related protein PR-1 precursor			CF653559	AAK30143	13,6	53,4
Putative disease resistance protein		+	CX675562	AAD20706	nd	9,2
Hypersensitive-induced response protein			CV718780	XP_476016	5,3	18,1
Putative nodulin		+	CV710110	AAN31815	nd	10,9
Putative protein serine/threonine kinase			CX308038	EAL71975	5,5	21,4
Light-regulated genes						
Chlorophyll A-B binding family protein	+		CF833152	NP_188923	3,2	nd
Early light-induced genes	+		CK937268	AAO33591	3,9	nd

* Additional binding sites for PthA1 and PthC2

Pereira, Tab. 3

Table 3. Main *Citrus sinensis* genes modulated by PthC1 and 2 with fold changes > 3, relative to controls (epicotyls expressing GUS), identified by microarray analysis. Down regulation is indicated by "-". Non-detected genes are indicated by "nd". Predicted PthC1 and 2-binding sites found in the respective *Citrus clementine* promoters are indicated by "+".

Target Description	PthC sites		Citrus	Target	Fold Change	
Auxin and GA signaling	**PthC1**	**PthC2**	**EST**	**Gene ID**	**PthC1**	**PthC2**
GH3 indole-3-acetic acid amido synthetase		+	CV714093	AAC61292	nd	-10,1
Nt-gh3 deduced protein	+	+	CF837666	AAD32141	-6,8	-6,5
Aux/IAA protein			CV704184	CAC84706	-5,1	-5,7
Aux/IAA protein	+		CV713157	CAC84706	-3,8	-4,0
Auxin-regulated protein			CF828380	AAG48763	-4,9	-6,0
Auxin-responsive protein		+	CK933306	NP_177688	-3,9	-3,8
Gbiaa-Re; auxin-regulated protein	+		CX674585	AAQ74955	-5,6	-6,0
IAA16 protein	+		CK701644	CAD30274	-5,7	-7,4
Nt-iaa4.5 deduced protein			CK934325	AAD32145	-3,3	-3,6
Auxin-regulated protein	+	+	CV714093	AAC61292	-9,2	-3,5
bZIP protein HY5			CN182471	NP_568246	-3,2	-3,9
bZIP transcription factor family protein			CF829107	NP_568457	-6,7	-6,2
CONSTANS-like protein	+	+	CK936954	AAG24863	-4,9	-5,0
Defense response / secondary metabolism						
S-adenosyl-L-homocysteine hydrolase		+	CK936768	NP_193130	nd	11,2
Caffeic acid O-methyltransferase			CX673755	2119166A	3,1	nd
Cinnamate 4-hydroxylase CYP73			CK936888	AAF66066	nd	3,6
Prephenate dehydratase family protein			AU186271	NP_19005	nd	5,5
Cinnamoyl-CoA reductase	+		CN187357	NP_180917	-5,4	-5,2
Cytochrome P450 monooxygenase		+	CF834243	O48922	-3,9	-4,1
Acridone synthase			CX665191	CAC14058	-4,2	-4,0
Pathogenesis-related protein PR1		+	CF653559	AAK30143	-6,4	-6,0
NAC domain protein			DN617664	CAC42087	nd	3,1
Ethylene responsive factor ERF			CK939541	O80337	nd	4,1
DNA binding protein S25-XP1 – AP2/ERF			CX043799	T03927	3,5	4,3
Cisteine proteinase	+	+	CX299481	BAC42063	-3,4	-3,5
Avr9/Cf-9 rapidly elicited protein 146	+		CX544652	AAG43551	3,2	nd
Light-regulated genes						
Chlorophyll A-B binding protein (Cab-11)			CF418034	S14305	3,7	nd
Chlorophyll a/b-binding protein			CX676086	BAA253931	3,7	nd
Chlorophyll a/b-binding protein 5			CK937181	B34013	3,2	3,8
Chlorophyll A-B binding protein	+	+	CF833152	NP_188923	-5,0	-6,2
Plastocyanin chloroplast precursor			CF836107	P17340	3,6	nd
Photosystem I reaction centre subunit IV			CX300551	CAD29821	3,3	nd
NADPH-protochlorophyllide		+	CN183674	BAA210891	3,5	nd

oxidoreductase						
UPA22 homolog; Lir1 light-regulated protein	*	**	CX672012	ACV71021	4,1	4,0
Early light induced protein	+	+	CK937268	AAO33591	-5,4	-8,4

* and ** indicate binding sites for PthAs 2 and 4, respectively

EXPERIMENTOS COMPLEMENTARES REFERENTES AO CAPÍTULO I

Este tópico tem por objetivo apresentar de forma detalhada os experimentos que compõe o Capítulo I, assim como aqueles realizados no período, que complementam este trabalho.

Material vegetal e inoculação das plantas

Mudas sadias de laranja doce (*C. sinensis*) foram obtidas a partir de viveiros e acondicionadas em ambiente com condições controladas: fotoperíodo de 16 horas de luz e temperatura de 25 ± 3ºC.

O isolado 306 de *X. axonopodis* pv. *citri* foi crescido em meio de cultura LB sólido sem adição de NaCl, denominado portanto, LBON, durante 48 h a 28ºC. As bactérias foram então coletadas por raspagem e ressuspendidas em água estéril. Todas as suspensões foram preparadas com uma densidade de aproximadamente 10^9 unidades formadoras de colônias por mililitro (OD_{600nm}=0,6). As folhas de laranja doce foram artificialmente infiltradas com um seringa de 1 mL. As seguintes suspensões foram infiltradas: H_2O; H_2O + ciclohexemida (50 µM); *X. citri*; *X. citri* + ciclohexemida (50 µM). As folhas foram coletadas após 8 h de infecção para processamento do RNA.

Processamento de RNA total e mensageiro

O processamento do RNA total foi realizado com Trizol conforme recomendações do fabricante (Invitrogen). As folhas foram maceradas em nitrogênio líquido e solubilizadas em Trizol. O RNA total foi precipitado por cerca de 16 h a 4°C com LiCl e ressuspendido em H_2O-DEPC, novamente precipitado com 0,1 volume de NaAc 3 M e 3 volumes de EtOH 100% e finalmente lavado com EtOH 70%. Ao final da extração, o RNA total foi ressuspendido em H_2O-DEPC e estocado em freezer – 80°C.

O isolamento do RNA mensageiro foi realizado com o kit Fast Track da Invitrogen conforme recomendações do fabricante. O preparo do RNA mensageiro para hibridização dos chips foi realizado com o kit da Affymetrix seguindo as especificações do fabricante.

Microarranjos e aquisição dos dados

As amostras de RNA mensageiro foram hibridizadas em Chips de DNA de citros (Affymetrix, Santa Clara, CA) conforme as recomendações do fabricante. Cada Chip de DNA contém aproximadamente 33 mil sondas representando, portanto, genes transcritos de citros que podem ser analisados simultaneamente com relação a seus respectivos níveis de expressão.

Os Chips foram escaneados (Affymetrix Gene Chip Scanner 3000–7G) e analisados no software ArrayAssist (ArrayAssit x.5, Stratagene, USA) utilizando-se o algoritmo MAS5 para normalização e correção da linha base. Os tratamentos foram analisados sempre em comparação a um tratamento controle, que normalmente não inclui a presença da bactéria ou da expressão transiente da proteína em questão. Dados como genes presentes, induzidos ou reprimidos foram levados em consideração. Todos os genes com nível de expressão (*fold-change*) acima de 3 foram analisados. Cada experimento foi realizado a partir de duas réplicas biológicas.

PCR quantitativo (qPCR)

Durante as etapas preparatórias do RNA mensageiro para hibridização do Chip de microarranjo foi sintetizado cDNA, o qual foi utilizado para o qPCR com o objetivo de validar a expressão dos genes bem como validar a eficiência do tratamento com ciclohexemida. O qPCR foi realizado com *SYBR Green*, que é uma sonda que se intercala no DNA à medida que a dupla fita se forma oriunda da reação de PCR em curso. O *SYBR Green* emite fluorescência apenas quando associado ao DNA que é, portanto, detectada e quantificada.

Os níveis de expressão de dois genes foram monitorados por qPCR nos diferentes tratamentos: *pathogenesis-related protein 1.1* (*pr1*) e *elicitor inducible cytochrome P450* (Figura 6). A expressão do gene *pr1* se manteve exacerbada mesmo em folhas de laranja infiltradas com *X. citri* na presença de ciclohexemida (Xac-Ch), enquanto o *elicitor inducible cytochrome P450* apresentou níveis de expressão similares tanto na presença quanto na ausência de ciclohexemida (Xac). Este resultado sugere que a expressão do gene *pr1* não é afetada pela ciclohexemida indicando que o nível de expressão observado para

este gene se deve à presença da bactéria, sobretudo a ação de efetores em potencial. Este resultado também demonstra o tratamento com ciclohexemida como uma eficiente estratégia para identificar genes induzidos por fitopatógenos. É importante, ainda, salientar que neste caso, o qPCR valida os níveis de expressão encontrados nos microarranjos.

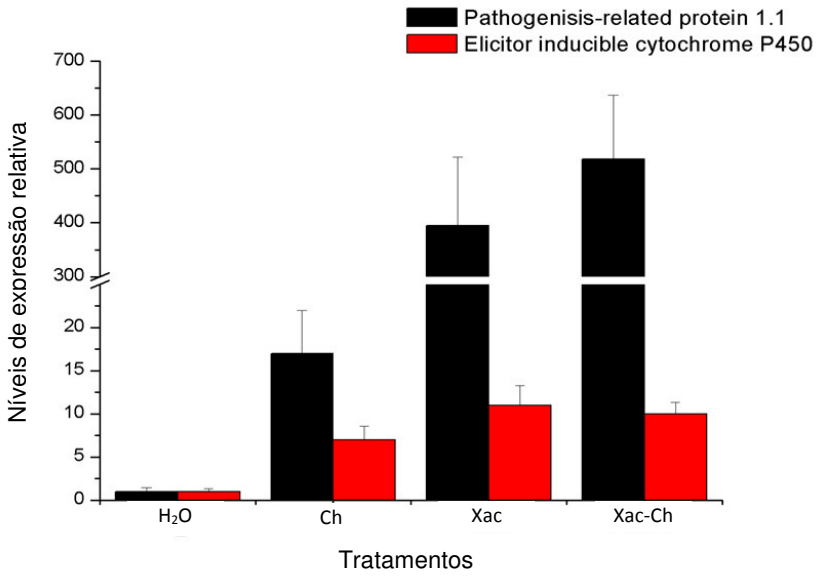


Figura 6. Análise por qPCR de genes induzidos nos microarranjos. Expressão dos genes *pathogenisis-related protein 1.1* e *elicitor-inducible cytochrome P450* em folhas de laranja doce infiltradas com H_2O, ciclohexemida (Ch), *X. citri* (Xac) e *X. citri* na presença de ciclohexemida (Xac-Ch).

Expressão e purificação de PthA recombinante com cauda 6xHis

O pré-inóculo da linhagem de *Escherichia coli* BL21(DE)pET28a-6xHis-PthA cresceu a 37°C por 12-16 h em LB acrescido com 50 µg/mL de canamicina. Depois o pré-inóculo foi diluído 1/100 em meio LB fresco com o referido antibiótico de seleção e incubado a 37°C/200 rpm. Após atingir a OD_{600} desejada (0,6–0,8) 0,4 mM de IPTG foi adicionado e a cultura incubada a 37°C por 5 horas e 200 rpm. As células foram coletadas por centrifugação, 6000 x g a 4°C por 10 minutos, e ressuspendidas no tampão A (20 mM de Tris-HCl pH 8,0, 5 mM de imidazol, 200 mM de NaCl, 1 mM de PMSF e 5% de glicerol). A reação de lise celular foi realizada com 0,3 mg/mL de lisozima e incubada no gelo por 30 minutos com agitação periódica. Realizou-se a sonicação com 6 pulsos de 20

segundos e 40% de amplitude (Sonic-Vibra Cell). O extrato total foi centrifugado a 17000 x g por 20 minutos a 4°C, filtrado através de membrana milipore de 0,45 µm e incubado a 4°C por 2 horas sob agitação lenta com um mix de DNase pancreática (1 µg/mL) e RNaseA (20µg/mL) e 100 µL de resina de afinidade por cobalto (TALON® Metal Affinity Resins) previamente equilibrada com tampão A. Três etapas de lavagens foram realizadas, uma com 10 volumes de tampão A e duas com 10 volumes de tampão B (tampão A com 10 mM de imidazol). A eluição foi realizada com tampão C (tampão A com 200 mM de imidazol). As eluições foram estocadas a 4°C e alíquotas foram estocadas em freezer – 80°C. Este protocolo foi padronizado para purificação de todas as variantes de 6xHis-PthA independente do volume de cultura, com o rendimento de frações com grau de pureza satisfatório (Figura 7).

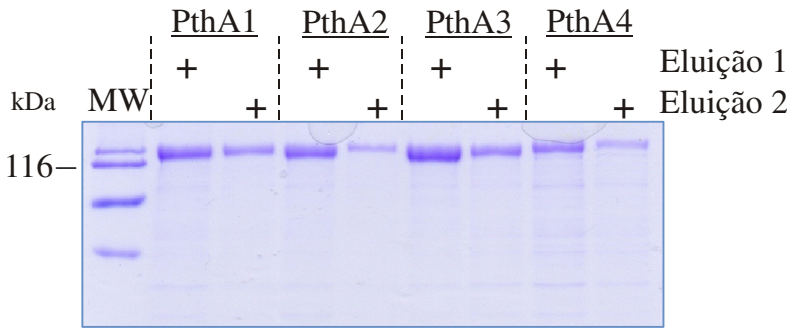


Figura 7. Purificação de PthA recombinante. Gel de poliacrilamida 10% com a resolução das proteínas recombinante PthA1-4 com cauda 6xHis purificadas por cromatografia de afinidade. MW: marcador de peso molecular.

Dicroísmo circular (circular dichroism ou CD) dos PthA

As quatro variantes de PthA com cauda 6xHis foram dialisadas em filtros amicon de 10 kDa. A membrana do filtro foi lavada/equilibrada duas vezes com 10 mL de tampão D (10 mM de Tris-HCl pH 8,0, 50 mM de NaCl, 2 % de glicerol e 1 mM de TCEP) sendo cada lavagem acompanhada de centrifugação 3000g a 4°C por 20 minutos ou até passar todo o volume de tampão. A fração da proteína (~1 mL) foi então adicionada e lavada três vezes com 10 volumes de tampão D. Após as lavagens as proteínas foram ressuspendidas em 1ml de tampão D e mantidas a 4°C. O dicroísmo circular de cada proteína foi realizado

com base em 20 acumulações, sendo que o espectro final é resultado da média das acumulações. Os dados foram normalizados de acordo com a elipticidade molar de cada proteína.

Após a purificação, as proteínas recombinantes apresentam estrutura secundária definida. Os espectros sugerem estrutura secundária bem definida para todas as variantes de PthA, predominantemente em α-hélice (Figura 8). No momento, não há nenhuma estrutura depositada no PDB (Protein Data Bank), no entanto, tanto a modelagem do AvrBs3 (Schornack *et al.*, 2006) quanto análises por Ressonância Magnética Nuclear (*nuclear magnetic resonance* ou RMN) de um peptídeo (1,5 resíduos de aminoácidos) do domínio interno de PthA e estudos espectroscópicos do domínio interno do PthA2 (Murakami *et al.*, 2010) sugerem uma estrutura secundária majoritariamente organizada em α-hélices. Da mesma forma, em consistência com esses dados, um software de predição aponta estrutura secundária predominantemente em α-hélices para o domínio central de PthA4 (Figura 9).

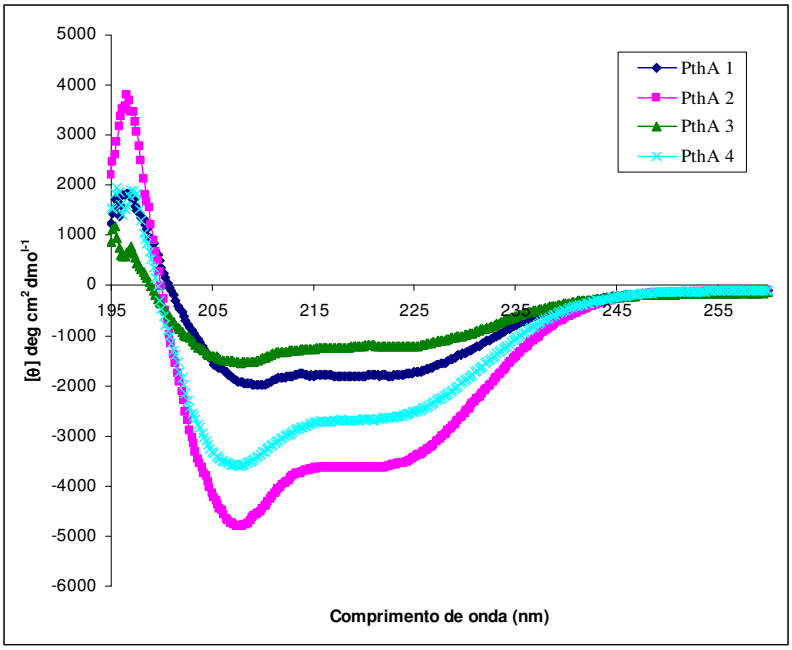


Figura 8. Comparação dos espectros de dicroísmo circular das quatro variantes de 6xHis-PthA. Os dados foram normalizados de acordo com a elipticidade molar de cada proteína. Os duplos mínimos em 222 e 208-210nm seguidos de um pico positivo em 196 nm sugerem estrutura secundária predominantemente em α-hélice para todas as isoformas. PthA2 (rosa) e 4 (turquesa) são semelhantes e estão mais bem estruturados que os PthA1 (azul) e 3 (verde), que também apresentam semelhanças estruturais entre si.

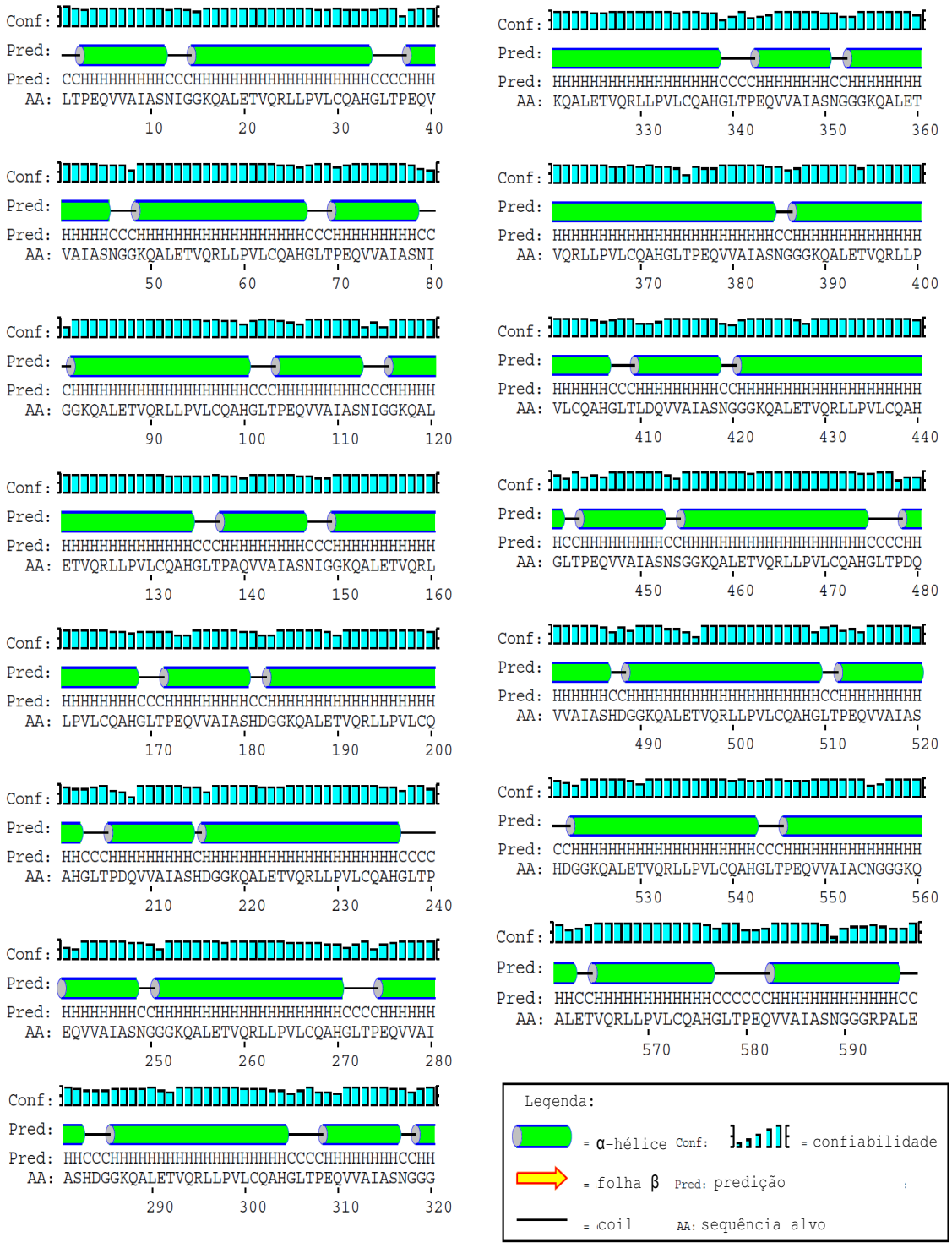


Figura 9. Predição de estrutura secundária do domínio central de PthA4. A predição foi realizada através do software PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/). O domínio repetitivo é formado basicamente por α-hélices. Nenhuma folha β foi identificada.

Biblioteca de DNA genômico de citros e amplificação de promotores

Uma biblioteca de DNA genômico foi construída para realizar a amplificação de promotores de citros utilizando-se iniciadores (*primers*) específicos desenhados a partir das etiquetas de sequências expressas (e*xpressed sequence tag* ou ESTs) selecionados nos microarranjos. A figura10 ilustra a estratégia utilizada para a construção da biblioteca.

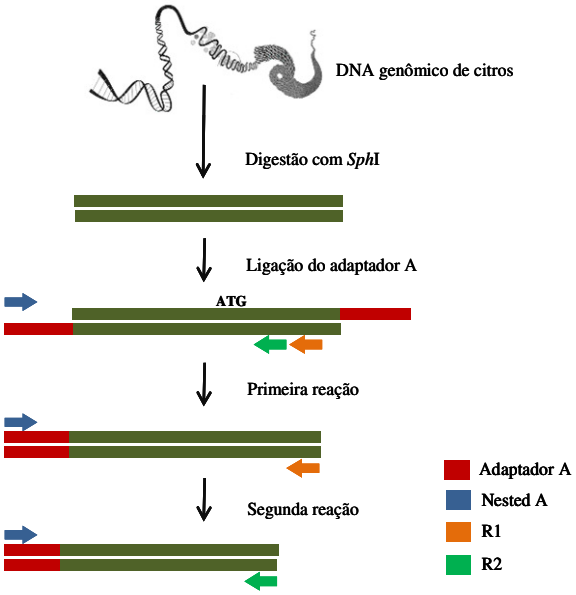


Figura 10. Estratégia utilizada para amplificar regiões promotoras de citros. O DNA genômico de laranja doce foi clivado com *Sph*I, gerando fragmentos de aproximadamente 500 pb a 2 kb, e subsequentemente ligada ao adaptador A, o qual contem o sítio para anelamento do iniciador Nested A. Os primers R1 e R2 foram sintetizados para anelar na porção 3´ adjacente ao ATG que dá início a tradução e amplificar a porção 5´ não codante do gene em combinação com o *Nested* A, em etapas de PCR *Nested*.

O sítio de restrição da enzima *Sph*I é GCATG·C (o ponto é referente ao local de clivagem), portanto produz uma porção dos fragmentos que podem conter um promotor ou sequência 5' não traduzida em potencial. Para o PCR a seguinte condição foi utilizada: 1 µL de DNA da biblioteca de citros diluída 1:100, 2 µL de tampão de PCR 10 x, 0,5 µL de $MgCl_2$ 50 mM, 0,5 µL de dNTPs 10 µM, 1,5 µL de *Nested* A 5 µM, 0,2 µL de Taq Platinum, volume final de 20 µL acertado com H_2O MiliQ autoclavada. A descrição dos oligonucleotídeos utilizados neste trabalho encontra-se na Tabela 2.

Dentre 15 genes selecionados a partir dos microarranjos, 3 regiões promotoras foram isoladas com sucesso neste trabalho referente aos seguintes genes: *cyclophilin* (*cyp*, EST CX299605), *chitinase* CHI1 (*chit*, EST CX663308) e *WRKY-type DNA binding protein* (wrky, EST CX046706). Outras duas regiões promotoras, referente aos genes *pathogenesis-related protein 5* (*pr5*, EST CK935296) e *pathogenesis-related protein PR-1* precursor (*pr1*, EST CF653559), foram isoladas pelo aluno de doutorado Andrés Cernadas, ex-membro do grupo. No entanto, estas proteínas também aparecem up-reguladas, inclusive no tratamento com ciclohexemida.

Tabela 2. Oligonucleotídeos utilizados para amplificação de promotores e EMSA.

Nome[a]	Sequência
chitinase CHI1-R1	5´ CTGGGGAGTCACAATGCTAGC 3´
chitinase CHI1-R2	5´ GGCAAGAATGCCCACAAGAGC 3´
putative cyclophilin-R1	5´ GTGACATCGGCGAAGAGCTCC 3´
putative cyclophilin-R2	5´ GACGGTCATGTCGAAGAACAC 3´
putative WRKY-type DNA binding protein-R1	5´ GACGGATCAGGTGCTCCATGC 3´
putative WRKY-type DNA binding protein-R2	5´ CCGTCTGATCTTCCATCATCC 3´
pathogenesis-related protein 5.2-R1	5´ AGCCCAGACCGTGTAGGGGC 3´
pathogenesis-related protein 5.2-R2	5´ CGAAAGTGGCTGCGTTGACCC 3´
pathogenesis-related protein PR-1 precursor-R1	5´ GCCAACACCAACCTGTGCCC 3´
pathogenesis-related protein PR-1 precursor-R2	5´ CATGGGAAGAGAGAATTAGGG 3´
PR5-*Box*-F	5´ TACACATTCTAAAATTTATATAAACCCTCATCCATTTCC 3´
PR5-*Box*-R	5´ GGAAATGGATGAGGGTTTATATAAATTTTAGAATGTGT 3´
PR5-*Up*-F	5´ TCAACTCTCAAAAATGTCTACAATACTAATCCAATCTAACAG 3´
PR5-*Up*-R	5´ CTGTTAGATTGGATTAGTATTGTAGACATTTTTGAGAGTTG 3´
PR5-*Down*-F	5´ TCCAAACATAGCCAAAATGAACTACTTTGTATCTTTATTA 3´
PR5-*Down*-R	5´ TAATAAAGATACAAAGTAGTTCATTTTGGCTATGTTTGG 3´
Nested A	5´ GATCTGCACCAGAATTCCATG 3´
Adaptor A	5´ TAATACGACTCACTATAGGATCTGCACCAGAATTCCATG 3´
Comp1-F	5´ TACACACCCACACCACCT 3´
Comp1-R	5´ AGGTGGTGTGGGTGTGTA 3´
Comp2-F	5´ TACACACCTCTTTTAAT 3´
Comp2-R	5´ ATTAAAAGAGGTGTGTA 3´
Comp3-F	5´ TACACATCTTTAAAACT 3´
Comp3-R	5´ AGTTTTAAAGATGTGTA 3´
Comp4-F	5´ TACAAACCTCTTTTACCTT 3´
Comp4-R	5´ AAGGTAAAAGAGGTTTGTA 3´
PR5-Comp2-F	5´ TACACATTCTAAAATTTACACACCTCTTTTAATATTTCC 3´
PR5-Comp2-R	5´ GGAAATATTAAAAGAGGTGTGTAAATTTTAGAATGTGT 3´
PR5-Comp4-F	5´ TACACATTCTAAAATACAAACCTCTTTTACCTTATTTCC 3´
PR5-Comp4-R	5´ GGAAATAAGGTAAAAGAGGTTTGTATTTTAGAATGTGT 3´

[a]R1 e R2: *primers* desenhados para amplificar promotores utilizando *Nested* PCR. F e R: *primers forward* e reverso, respectivamente.

A análise de regiões promotoras de citros

A Figura 11 demonstra as análises das regiões promotoras amplificadas, as quais foram realizadas através de dois programas "caça-promotor" distintos: o PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) e o PROSCAN (http://www-bimas.cit.nih.gov/molbio/proscan/). O primeiro é uma base de dados que reúne promotores de plantas juntamente com seus elementos "*cis*" regulatórios. O segundo é um programa de predição que identifica promotores com base na homologia entre sequências alvo da RNA polimerase II de eucariotos.

A >PR5 452nt

```
+ ATCTGCACCA GAATTCCATG CATTCATTCT AGTGAAATTT TCTATATCTT AATCTTAATT GCTAAGTTAA
- TAGACGTGGT CTTAAGGTAC GTAAGTAAGA TCACTTTAAA AGATATAGAA TTAGAATTAA CGATTCAATT

+ AATAACCCAC ATAAGCTTCC AACGAGGGTG ATTTAAGTCA ACACCACGAG AATTATTAAT TAGTGACGAC
- TTATTGGGTG TATTCGAAGG TTGCTCCCAC TAAATTCAGT TGTGGTGCTC TTAATAATTA ATCACTGCTG

+ GGACAACCAC CGACGGCAAT GCTACATATT AATACTAAAT AATTAAATAA ATTTCACATA TAGAATCGGT
- CCTGTTGGTG GCTGCCGTTA CGATGTATAA TTATGATTTA TTAATTTATT TAAAGTGTAT ATCTTAGCCA

+ CTCATATATT TGCTAGCTAC ATTTCTTGAC CAATCTTCAT ATGGTGCCAA ACACCACCAC ATCAACTCTC
- GAGTATATAA ACGATCGATG TAAAGAACTG GTTAGAAGTA TACCACGGTT TGTGGTGGTG TAGTTGAGAG

+ AAAAATGTCT ACAATACTAA TCCAATCTAA CAGTACACAT TCTAAAATTT ATATAAACCC TCATCCATTT
- TTTTTACAGA TGTTATGATT AGGTTAGATT GTCATGTGTA AGATTTTAAA TATATTTGGG AGTAGGTAAA

+ CCCCTCCAAA CATAGCCAAA ATGAACTACT TTGTATCTTT ATTAAATGCA TCCTTCCTTT TTCTCACCCT
- GGGGAGGTTT GTATCGGTTT TACTTGATGA AACATAGAAA TAATTTACGT AGGAAGGAAA AAGAGTGGGA

+ GTACTTAACT TGGGTCAACG CAGCCACTTT C
- CATGAATTGA ACCCAGTTGC GTCGGTGAAA G
```

B >PR1 480nt

```
+ GTAGTTAAAC AATTTAATGT GGAGGATTAA ATATTATGTT ATCTTAAGAA ACCAAATTCT AATGATTAAT
- CATCAATTTG TTAAATTACA CCTCCTAATT TATAATACAA TAGAATTCTT TGGTTTAAGA TTACTAATTA

+ TCTTTTCCCA TTTCATGTCG TTAGCTATAG TAATCTATAA GTCACGACTT CCTAAAAATG AAACAAAACG
- AGAAAAGGGT AAAGTACAGC AATCGATATC ATTAGATATT CAGTGCTGAA GGATTTTTAC TTTGTTTTGC

+ TGGAAATTGA TTTTGATTGG ATCAGAAGAG TAAGCTACAA ATCAACTGTA TCTTATTAGA AATTGAGCTG
- ACCTTTAACT AAAACTAACC TAGTCTTCTC ATTCGATGTT TAGTTGACAT AGAATAATCT TTAACTCGAC

+ AAGGTGACGA CAATAGTGTA ATTTTCTTTC ATTTGTACTA GATTTTTTTC ATTCTCAAGT TCCCTAAACC
- TTCCACTGCT GTTATCACAT TAAAAGAAAG TAAACATGAT CTAAAAAAAG TAAGAGTTCA AGGGATTTGG

+ ACTACGCAAT CTTGTTTTTG AACATTCTTC TTAATTTGCC TAATTTTCTT CCAACCGTTA TCTCTATAAA
- TGATGCGTTA GAACAAAAAC TTGTAAGAAG AATTAAACGG ATTAAAAGAA GGTTGGCAAT AGAGATATTT

+ TACCAGTCGT ACCATCCCAT TTTTTCATCA ATCATCTTGC AAAATCTTTA ATCACAAAAT TTGCAAAACA
- ATGGTCAGCA TGGTAGGGTA AAAAAGTAGT TAGTAGAACG TTTTAGAAAT TAGTGTTTTA AACGTTTTGT

+ AAAAGACAAA TGGGGTTTTC CAAGAATTCA TCACTACCTC TTTTTTGTAT CTTGGGGTT
- TTTTCTGTTT ACCCCAAAAG GTTCTTAAGT AGTGATGGAG AAAAAACATA GAACCCCAA
```

C

>Chit1 577nt

```
+ GTGTGTGTAT TTATATAAAC AAAGTGGGTA GACAATTAAA AATAAACATG ACTGAATGTC AATATCCGAA
- CACACACATA AATATATTTG TTTCACCCAT CTGTTAATTT TTATTTGTAC TGACTTACAG TTATAGGCTT

+ AGCTCAAAAC AAGTATGAGC TTAAGATTTT CCAATTTACA ATTAATTCAA CTACAATAAA ATAAATAAAT
- TCGAGTTTTG TTCATACTCG AATTCTAAAA GGTTAAATGT TAATTAAGTT GATGTTATTT TATTTATTTA

+ TTACCTCAAA ATTTACTGAA AATTAATTAT GTAAAATTAT ATTTTGTATA ATTTTTATTC AAATTTAATT
- AATGGAGTTT TAAATGACTT TTAATTAATA CATTTTAATA TAAAACATAT TAAAAATAAG TTTAAATTAA

+ TGAGAATTTT TAATATATAT GGCGGTCAAT CAACAACCCA CTCGAAAGAC TTAATTTATT TTCTTTTATT
- ACTCTTAAAA ATTATATATA CCGCCAGTTA GTTGTTGGGT GAGCTTTCTG AATTAAATAA AAGAAAATAA

+ TTATTTCTAG TTTTTCTGAC AAGTCAAGCT GGCTAGATTT CAAGCCACAA CAAATGCGAA GTCTAGAAAA
- AATAAAGATC AAAAAGACTG TTCAGTTCGA CCGATCTAAA GTTCGGTGTT GTTTACGCTT CAGATCTTTT

+ ACCTATCAGA GATAAATTCT ATGTTCAAAT TTATCACAAT TCACCAGTAC GTGGCAGAAA TAACGTCCAT
- TGGATAGTCT CTATTTAAGA TACAAGTTTA AATAGTGTTA AGTGGTCATG CACCGTCTTT ATTGCAGGTA

+ TTTGCACTCT ATAAATACCG TACTCCCATC ACCAACATTT GCATATCACT CACATTTCTC ATTACTTCTT
- AAACGTGAGA TATTTATGGC ATGAGGGTAG TGGTTGTAAA CGTATAGTGA GTGTAAAGAG TAATGAAGAA

+ AAATTACTCC GTGAACTTTC CCTCAAAATG GCTCCACTTA TTATGAGAAA AAATATTGTA ACCTTTGCTC
- TTTAATGAGG CACTTGAAAG GGAGTTTTAC CGAGGTGAAT AATACTCTTT TTTATAACAT TGGAAACGAG

+ TTGTGGGCAT TCTTGC
- AACACCCGTA AGAACG
```

D

>wrky50 617nt

```
+ TTTATTAAAT AATGAGAGAT AAAGAGAGAG GAAAATATAA AGAAAGGATT TCCAACGTAT ATATTTAATT
- AAATAATTTA TTACTCTCTA TTTCTCTCTC CTTTTATATT TCTTTCCTAA AGGTTGCATA TATAAATTAA

+ AAAAATGCGC TATAAAACAT GCAAATTACA GTGATTATAT ACGGAGTTTG AACTTGGCAA AGTCGCTCTT
- TTTTTACGCG ATATTTTGTA CGTTTAATGT CACTAATATA TGCCTCAAAC TTGAACCGTT TCAGCGAGAA

+ CGTTTTGTCA AACACATTGA AAATTCAAAA CCAAAAAGCA CTCCTAAGAC CCCGATAATA CTTTTTCCAC
- GCAAAACAGT TTGTGTAACT TTTAAGTTTT GGTTTTTCGT GAGGATTCTG GGGCTATTAT GAAAAAGGTG

+ AGGGTCCCCA CCACCCACAT ACTAATATTT GTTACTTTTT TAAAATTCAA ACAAGACCAA CCTCTGATTT
- TCCCAGGGGT GGTGGGTGTA TGATTATAAA CAATGAAAAA ATTTTAAGTT TGTTCTGGTT GGAGACTAAA

+ GTTTAATCAC CATAGAAATC GTCAAACCAC TCTGAACCGT TGGATCAAAA CCCCACGCTT TGACTAAACT
- CAAATTAGTG GTATCTTTAG CAGTTTGGTG AGACTTGGCA ACCTAGTTTT GGGGTGCGAA ACTGATTTGA

+ CACCCTCTCC CGGTCATCCG GTCACCCCCA CTTCCTCTAT AATATCTTCC CCACCCCTGT ATCTTCTCTC
- GTGGGAGAGG GCCAGTAGGC CAGTGGGGGT GAAGGAGATA TTATAGAAGG GGTGGGGACA TAGAAGAGAG

+ AACGATTCAA GGAAGAGAGA GAGCCTCCAC CCTCGCAGCC TCAGCCTTAA GTTTTACTTT TTCAAAGGGT
- TTGCTAAGTT CCTTCTCTCT CTCGGAGGTG GGAGCGTCGG AGTCGGAATT CAAAATGAAA AAGTTTCCCA

+ TTCGTCGAAT GACATCTTGA GGCTTTTCCA AATTGCTCTC CTTTGGTAGC AGAAACTTTT TCGTTTTTCT
- AAGCAGCTTA CTGTAGAACT CCGAAAAGGT TTAACGAGAG GAAACCATCG TCTTTGAAAA AGCAAAAAGA

+ AAATCATGGC GGTGGAGCTG ATGGGATTTC CAAAAAGGAT GATGGAAGAT CAGACG
- TTTAGTACCG CCACCTCGAC TACCCTAAAG GTTTTTCCTA CTACCTTCTA GTCTGC
```

E

Proscan: Version 1.7
Processed Sequence: 1185 Base Pairs

```
Promoter region predicted on forward strand in 815 to 1065
Promoter Score: 61.72 (Promoter Cutoff = 53.000000)
TATA found at 1049.  Est.TSS = 1079
Significant Signals
```

Name	TFD #	Strand	Location	Weight
HSV_IE_repeat	S01565	-	824	1.363000
AP-2	S01936	+	970	1.108000
EARLY-SEQ1	S01081	+	970	6.322000
(Sp1)	S01187	+	970	8.117000
Sp1	S00801	+	971	2.755000
JCV_repeated_sequenc	S01193	-	972	1.658000
Sp1	S00802	+	972	3.292000
Sp1	S00781	-	976	2.772000
Sp1	S00978	-	977	3.361000
Sp1	S00327	-	978	17.211000
Sp1	S00064	-	978	5.934000
Sp1	S01542	-	978	3.608000
Sp1	S00979	-	978	6.023000
AP-2	S00346	+	1030	1.355000

Figura 11. Busca por elementos de regulação transcricional em regiões promotoras de citros. (**A-D**) PLANTCARE indica os elementos TATA na dupla fita de DNA com um grifo colorido. O TATA-*box* de relevância para este estudo está indicado por um circulo vermelho. (**E**) O PROSCAN por sua vez indica a posição do elemento TATA encontrado (seta amarela).

A análise *in silico* das sequências promotoras revelou a presença de um TATA-*box* comum e notavelmente semelhante ao encontrado no gene *upa20* de pimentão, denominado *UPA-box* (Figura 12, em cinza). Os TATA-*box* foram escolhidos levando-se em consideração o "*score*" dado pelo software e pela proximidade ao ATG. Particularmente a sequência que exibe maior grau de similaridade com o *upa20* é a do gene *pr5*.

```
upa20    TTTCCTATCTCTCTCTTCATCTTTATATAAACC-TGACCCTTTGTGACATTC   52
pr5      CTAACAGTACACATTCTAAAATTTATATAAACCCTCATCCATTTCCCCTCCA   52
          *  *  *     *  * *  ************ * * ** **    *

upa20    TTTCCTATCTCTCTCTTCATCTTTATATAAACCTGACCCTTTGTGACATTCT   52
pr1      AATTTTCTTCCAACCGTTATCTCTATAAATACCAGTCGTACCATCCCATTTT   52
           *  * *  *   * * **** **** * *** * *      *  **** *

upa20    TTTCCTATCTCTCTCTTCATCTTTATATAAACCTGACCCTTTGTGACATTCT   52
cyp      TTACTCCCCCACCGCTTCATCCCC-TATTTAATTGCCCCAAAACCCTAACAA   51
         ** *    *   * *******    ***  *  ** ***        *

upa20    --TTTCCTATCTCTCTCTTCATCTTTATATAAACCTGACCCTTTGTGACATTCT 52
chit1    CAGAAATAACGTCCATTTTGCACTCTATAAATACCGTACTCCCATCACCAAC-- 52
                *  **  * **   ** **** * ***  ** *       **

upa20    TTTCCTATCTCTCTCTTCATCTTTATATAAACCTGACCCTTTGTGACATTCT-  52
wrky50   CATCCGGTCACCCCCACTTCCTCTATA-ATATCTTCCCCACCCCTGTATCTTC  52
           ***  ** * * *     ** **** * * **  ***        **  *
```

Figura 12. Análise *in silico* das sequências promotoras dos genes *pr5, pr1, cyp, chit2 e wrky50*. As sequências contendo a região do TATA-*box* foram comparadas através do software Clustal W (http://www.ebi.ac.uk/Tools/clustalw2/index.html). Em negrito está o TATA-*box* identificado através dos softwares PlantCARE ou PROSCAN em comparação com o *UPA-box* (em cinza) encontrado no *upa20* ligação. Os nucleotídeos sublinhados não são conservados. Em azul está a sequência consenso entre a *cyp* e o *upa20*. Os asteriscos indicam os nucleotídeos similares.

Recentemente, Kay *et. al* (2007) descreveram a proteína de avirulência AvrBs3 como um fator de transcrição que liga direta e especificamente o *UPA-box* desse gene, que é por sua vez o regulador majoritário do processo de hipertrofia causado por *X. vesicatoria*. O fato de PthA apresentar cerca de 96% de similaridade com AvrBs3 (Schornack *et al.*, 2006), juntamente com o isolamento de sequências de citros contendo elementos semelhantes ao *UPA-box*, encorajaram os experimentos de ensaio de retardamento de mobilidade eletroforética (*electrophoretic mobility shift assay* ou EMSA). Além disso, as sequências estudadas são ricas em 'AT' que são preferencialmente ligadas por efetores da

família AvrBs3/PthA. Vale, ainda, ressaltar a presença de uma região similar entre o *upa20* e a *cyp* localizada a montante do TATA-*box* (Figura 12, em azul). Essa sequência não foi identificada como um sítio de ligação propriamente dito, também não apresentou similaridade com outros sítios de ligação preditos pelos softwares utilizados, entretanto ela pode desempenhar papel importante para a ligação de PthA.

Transformação transiente de citros mediada por Agrobacterium tumefaciens

Epicótilos de laranja doce 'Hamlin' foram transformados independentemente com o vetor pBI121 vazio, pBI121/P2 (construção com PthA2 inteiro) e pBI121/P4 (construção com PthA4 inteiro) utilizando o sistema mediado por *A. tumefaciens*. Os vetores pBI121/P2 e pBI121/P4 não levam o gene repórter GUS. A suspensão de *A. tumefaciens* de OD_{600} entre 0,8-1 foi incubada com os epicótilos por 15 minutos na presença de 100 µM acetoceringona. Para aumentar a eficiência de transformação, os epicótilos foram feridos e seccionados longitudinalmente. Em seguida os epicótilos foram secos em papel de filtro e transferidos para meio MS semi-sólido fresco acrescido de 100 µM acetoceringona, em seguida foram mantidos no escuro por 72 horas.

Ensaio histoquímico de atividade da β-glucuronidase.

Com base no protocolo descrito por Jefferson *et al.* (1987) com algumas alterações. Após o co-cultivo apenas os explantes transformados com o vetor pBI121 vazio, contendo o repórter GUS (gene que codifica a enzima β-glucuronidase), foram incubados a 37°C por ~24 horas com 1ml do tampão X-Gluc (1 mM de X-Gluc [ácido 5-bromo-4-choloro-3-indolil glucuronídeo], 50 mM de Na2HPO4 pH 7,0, 0,1% Triton X-100, 1 mM de EDTA e 10 mM de DTT) para verificar a atividade da enzima. O material foi diafanizado em EtOH 70%.

CAPÍTULO II: Estudos de interação PthA-DNA *in vivo*.

Neste ultimo capítulo serão apresentados experimentos realizados para verificar a ligação de PthA *in vivo* a potenciais alvos isolados a partir de citros. As regiões promotoras destes genes foram analisadas através da imunoprecipitação de cromatina e da co-transformação mediada por *A. tumefaciens*, a fim de verificar se a proteína PthA era capaz de modular a transcrição de tais genes *in vivo*.

Na região promotora destes genes identificou-se a presença de elementos TATA-*box* notavelmente semelhantes ao *UPA-box*, encontrado no *upa20*, um gene de pimentão que é regulado por AvrBs3, uma proteína homóloga a PthA. Esse resultado levou à hipótese de que PthA poderia exibir atividade de ligação por estas sequências, encorajando portanto, a realização dos experimentos descritos acima.

INTRODUÇÃO

As proteínas relacionadas à patogênese (*pathogenesis-related proteins* ou PRs) são proteínas ácidas com atividade antimicrobiana que se organizam em 17 famílias (Tabela 3). De maneira geral, as PRs são induzidas mediante a estresses bióticos e abióticos. A expressão destas proteínas ocorre durante o desenvolvimento vegetal, em resposta a ferimentos e a baixas temperaturas, algumas delas demonstrando até atividade contra o congelamento, além de participarem nas respostas de defesa contra patógenos. Assim, diante desta comprovada multi-funcionalidade, fica claro que estas proteínas ocupam lugar de destaque para o desenvolvimento da planta (van Loon *et al.*, 2006; Campos *et al.*, 2007).

Tabela 3. Famílias de proteínas PRs. Adaptado (van Loon, Rep *et al.*, 2006).

Family	Type member	Properties	Gene symbols
PR-1	Tobacco PR-1a	Unknown	*Ypr1*
PR-2	Tobacco PR-2	β-1,3-glucanase	*Ypr2*, [*Gns2* (‘*Glb*’)]
PR-3	Tobacco P, Q	Chitinase type I, II, IV, V, VI, VII	*Ypr3*, *Chia*
PR-4	Tobacco ‘R’	Chitinase type I, II	*Ypr4*, *Chid*
PR-5	Tobacco S	Thaumatin-like	*Ypr5*
PR-6	Tomato Inhibitor I	Proteinase-inhibitor	*Ypr6*, *Pis* (‘*Pin*’)
PR-7	Tomato P_{69}	Endoproteinase	*Ypr7*
PR-8	Cucumber chitinase	Chitinase type III	*Ypr8*, *Chib*
PR-9	Tobacco “lignin-forming peroxidase”	Peroxidase	*Ypr9*, *Prx*
PR-10	Parsley “PR1”	Ribonuclease-like	*Ypr10*
PR-11	Tobacco “class V” chitinase	Chitinase, type I	*Ypr11*, *Chic*
PR-12	Radish Rs-AFP3	Defensin	*Ypr12*
PR-13	Arabidopsis THI2.1	Thionin	*Ypr13*, *Thi*
PR-14	Barley LTP4	Lipid-transfer protein	*Ypr14*, *Ltp*
PR-15	Barley OxOa (germin)	Oxalate oxidase	*Ypr15*
PR-16	Barley OxOLP	Oxalate-oxidase-like	*Ypr16*
PR-17	Tobacco PRp27	Unknown	*Ypr17*

As PRs são induzidas através da sinalização de compostos tais como: ácido salicílico, ácido jasmônico ou etileno os quais estão intimamente relacionados a respostas de defesa induzidas por fitopatógenos. O termo do inglês ‘*pathogenesis-related*’ refere-se ao fato destas proteínas serem induzidas de forma associada à respostas de defesa em geral,

no entanto, isso não implica um papel por si só nos processos de defesa vegetal (van Loon *et al.*, 2006).

Contudo, gradativamente a literatura relata a atividade destas proteínas contra diferentes patógenos e seus efeitos de proteção ou resistência. Algumas PRs apresentam propriedades que sugerem por si só papel na defesa, como no caso das endoquitinases das famílias PR-3, 4, 8 e 11, que poderiam atuar contra fungos em função da atividade de degradação da quitina (Melchers *et al.*, 1994).

As PR-1 e 5, por sua vez, promovem à atividade contra omicetos. PR-1, por exemplo, foi detectada em zonas de abscisão podendo estar envolvida no remodelamento de parede celular ou atuar como componentes de defesa em ferimentos (Roberts *et al.*, 2000). *pr5* por sua vez é induzido por estresse osmótico e por patógenos. Verificou-se que PR-5 possui atividade anti-omiceto contra *Phytophthora infestans in vitro* e confere resistência a este patógeno em plantas transgênicas de tabaco (Velazhahan e Muthukrishnan, 2003). Além disso, PR-5 confere resistência a um número de cultivares, porém somente a determinados tipos de patógenos (van Loon *et al.*, 2006). É o caso da osmotina de tomate que confere à laranja transgênica, resistência a *P. citrophthora* (Fagoaga *et al.*, 2001). Além disso, outras funções já foram relatadas para PR-5, tais como: permeabilizadores de membrana (Anzlovar e Dermastia, 2003), ligação e hidrólise de glucanos (Grenier *et al.*, 1999) e apoptose (Ibeas *et al.*, 2000).

Finalmente, o fato de algumas PRs estarem envolvidas com o remodelamento de parede celular e respostas de defesa, em especial PR-5, levou a uma investigação mais aprofundada sobre este candidato. Tanto *pr1* quanto *pr5* foram regulados em altos níveis de expressão 6-48 horas após a infecção de folhas de citros com *X. citri* (Cernadas *et al.*, 2008), e continuaram sendo reguladas mesmo na presença de um inibidor de síntese protéica, ciclohexemida, infiltrado juntamente com *X. citri* em folhas de citros. Além disso, as regiões promotoras dos genes que codificam estas proteínas apresentam um TATA-*box* que é semelhante ao *UPA-box* sendo ligado *in vitro* pelos PthA.

Neste capítulo descreve-se as tentativas de detectar PthA 2 e 4 na cromatina, associados ao promotor do gene *pr5* pela técnica de ChIP e de verificar a transativação do mesmo promotor *in vivo* por PthA4.

MATERIAIS E MÉTODOS

Material vegetal, infiltração, e plasmídeos

Mudas sadias de tabaco (*Nicotiana tabacum*) mantidas a condições controladas de fotoperíodo de 16 horas de luz e temperatura de 25 ± 3ºC foram utilizadas para o experimento de co-transformação. As cepas e plasmídeos descritos nesta seção estão descritos na Tabela 4. A bactéria *A. tumefaciens* foi cultivada em meio YEP sólido (acrescido dos antibióticos apropriados) a 28ºC durante 48 horas. As células foram coletadas por raspagem e inoculadas meio YEP líquido fresco e cultivadas nas mesmas condições até atingir a OD_{600nm}= 1. As células foram coletadas por centrifugação (8000 rpm/25ºC /10 minutos) e ressuspendidas em meio MS acrescido de acetoceringona (100 µM) preservando a OD_{600nm}= 1 para cada suspensão. As folhas foram removidas 24, 48 e 72 horas após a infiltração e submetidas ao ensaio de atividade da β-glucuronidase.

Tabela 4. Cepas e plasmídeos utilizados neste estudo.

Cepa/plasmídeo	Características	Referência
Escherichia coli		
DH5α	Estirpe de clonagem	BRL, Bethesda, MD
BL21 (DE3)	Estirpe de expressão heteróloga	Estoque particular
Xanthomonas citri		
306	Estirpe selvagem; Grupo A; afeta *Citrus* spp.	(da Silva *et al.*, 2002)
Agrobacterium tumefaciens		
EHA105	Estirpe para transformação genética de plantas;	
Plasmídeos		
pET-28a DNA	Vetor de clonagem e expressão; contém sequência de His•Tag no N-terminal e C-terminal, codifica *lacI*, resistência canamicina	Novagen, USA
pBI121	Vetor binário para transformação de plantas; contém gene repórter GUS e promotor CaMV 35S no T-DNA, resistência canamicina	(Chen *et al.*, 2003)
pGEM-T Easy	Vetor para clonagem de fragmentos de PCR; resistência ampicilina	Promega, USA
pET-28a-P2ΔRD	Vetor derivado do pET-28a DNA; contém PthA2 com apenas 1,5 repetição; resistência canamicina	Este estudo

Clonagem de PthA sem o domínio de repetição

O plasmídeo pBI121 contendo a sequência completa do *pthA2* foi clivado com *Xba*I e *Eco*RI produzindo a remoção da sequência inteira do *pthA2* mais a região Nos-ter. Este fragmento foi clonado no pET28a gerando o pET28a-*pthA2* que, em seguida, foi clivado com *Msc*I que digere entre as unidades de repetição. Após a remoção do domínio interno, o fragmento resultante foi novamente clonado no pBI121, gerando pET28a/*pthA2*ΔRD.

Transformação transiente de citros mediada por Agrobacterium tumefaciens

Epicótilos de laranja doce 'Hamlin' foram transformados independentemente com o vetor pBI121 vazio, pBI121/P2 (construção com PthA2 inteiro) e pBI121/P4 (construção com PthA4 inteiro) utilizando o sistema mediado por *A. tumefaciens*. Os vetores pBI121/P2 e pBI121/P4 não levam o gene repórter GUS. A suspensão de *A. tumefaciens* de OD_{600} entre 0,8-1 foi incubada com os epicótilos por 15 minutos na presença de 100 µM acetoceringona. Para aumentar a eficiência de transformação, os epicótilos foram feridos e seccionados longitudinalmente. Em seguida os epicótilos foram secos em papel de filtro e transferidos para meio MS semi-sólido fresco acrescido de 100 µM acetoceringona, sendo em seguida mantidos no escuro por 72 horas.

Ensaio histoquímico de atividade da β-glucuronidase.

Com base no protocolo descrito por Jefferson *et al.* (1987) com algumas alterações. Após o co-cultivo, apenas os explantes transformados com o vetor pBI121 vazio, contendo o repórter GUS (gene que codifica a enzima β-glucuronidase), foram incubados a 37°C por ~24 horas com 1ml do tampão X-Gluc (1 mM de X-Gluc [ácido 5-bromo-4-choloro-3-indolil glucuronídeo], 50 mM de Na2HPO4 pH 7,0, 0,1% Triton X-100, 1 mM de EDTA e 10 mM de DTT) para verificar a atividade da enzima. O material foi diafanizados em EtOH 70%.

Imunoprecipitação de cromatina (Chromatin immunopreciptation ou ChIP)

Com base no protocolo descrito por Bowler *et al.* (2004) com algumas alterações. Epicótilos de laranja doce com expressão transiente de *pthA2* ou *pthA4*, e epicótilos

transformados com vetor pBI121 vazio, como controle negativo, foram utilizados. Os epicótilos foram lavados com água destilada autoclavada e infiltrados a vácuo com 30 ml do tampão de MC (0,4 M de sucrose, 10 mM de Tris-HCl pH 8,0, 5 mM de β-ME, 0,1 de PMSF, 1 % de formaldeído e coquetel de inibidores de protease [Roche Diagnostics cat. 11 836 153 001]) por 20 minutos à temperatura ambiente (TA). Nesta etapa, o material é fixado pela adição do formaldeído ao tampão MC, que induz o *cross-linking* (ligações cruzadas). Em seguida, para interromper o *cross-linking*, 0,125 M de glicina foi infiltrada a vácuo por 10 minutos a TA. Os epicótilos foram então lavados duas vezes com água destilada autoclavada e congelados em nitrogênio líquido. Se necessário, nesta etapa o material pode ser armazenado em freezer –80°C.

Primeiramente, 50 µl de dynabeads (resina magnética com proteína A imobilizada, Invitrogen Ltda.) foi lavada 2 vezes: uma com 10 volumes de tampão PBS 2X acrescido de 0,1% de Triton X-100 e outra com o tampão ChIP (1,1% de Triton X-100, 1,2 mM de EDTA, 16,7 mM de Tris-HCl pH 8,0, 167 mM de NaCl, 1 mg/mL de BSA e 10 µg/mL salmon sperm DNA (SIGMA D-1626)). Novamente, a dynabeads foi ressuspendida em 50 µl de tampão ChIP fresco e desta vez incubada a 4°C sob rotação com o anti-PthA (anticorpo recombinante policlonal contra PthA2, produzido em coelho pela Célula B – Centro de Biotecnologia do Estado do Rio Grande do Sul (http://www.celulab.ufrgs.br/) na titulação 1:50 por 4-6 h. Durante o período de incubação da dynabeads, o material vegetal foi macerado em nitrogênio líquido, ressuspendido em 30 ml de tampão M1 (10 mM de fosfato de sódio pH 7,0, 0,1 M de NaCl, 1 M de 2-metil 2,4-pentanediol, 10 mM de beta-mercaptoetanol, 0,1 mM de PMSF, e coquetel de inibidores de protease [Roche Diagnostics cat. 11 836 153 001]) e filtrado por gravidade através de uma camada de Miracloth a 4°C. O filtrado foi centrifugado por 10 minutos a 1000 x g a 4°C e lavado 2 vezes com 2,5ml de tampão M2 (tampão M1 mais 10 mM de $MgCl_2$, 0,5 % Triton X-100), intercalando passos de centrifugação nas mesmas condições descritas acima, por apenas 5 minutos. Em seguida o sedimentado foi ressuspendido em 5ml de tampão M3 (10 mM de fosfato de sódio pH 7,0, 0,1 M de NaCl, 10 mM de beta-mercaptoetanol, 0,1 mM de PMSF e coquetel de inibidores de protease [Roche Diagnostics cat. 11 836 153 001]) e novamente centrifugado. O sedimentado nuclear foi ressuspendido em 300µl do tampão de lise (1 % de SDS, 10 mM

de EDTA e 50 mM de Tris-HCl pH 8,0) e solubilizado por sonicação (Diagenode Bioruptor: 10 minutos no modo '*Low*' e 5 minutos no modo médio). As amostras foram centrifugadas por 5 minutos a 4°C na rotação máxima. Cerca de 20µl foram separados como '*input*' e armazenados em freezer –20°C. Os 50 µl de dynabeads previamente incubados com o anti-PthA foram devidamente lavados com 10 volumes de tampão ChIP. As amostras foram então diluídas em 3 mL do tampão ChIP e incubadas com a dynabeads por cerca de 12 h a 4°C. Em seguida a resina foi lavada com 1ml dos seguintes tampões:

- 1x com Tampão salino A (*0,1% de SDS, 1% de Triton X-100, 2 mM de EDTA, 20 mM de Tris-HCl pH 8,0 e 150 mM de NaCl*)

- 1x com: Tampão salino B (*0,1% de SDS, 1% de Triton X-100, 2 mM de EDTA, 20 mM de Tris-HCl pH 8,0 e 500 mM de NaCl*)

- 1x com: Tampão LiCl (*0,25 M de LiCl, 1% de NP40, 1% de desoxicolato, 1 mM de EDTA e 10 mM de Tris-HCl pH 8,0*)

- 2x com: TE (*10 mM de Tris-HCl e 1 mM de EDTA pH 8,0*)

Duas etapas de eluição foram realizadas com 250 µl de tampão de eluição (1 % de SDS e 0,1 M de $NaHCO_3$) fresco cada à TA por 15 minutos. Tanto as amostras quanto os '*input*' foram incubadas por 4hs a 65°C com 200 mM de NaCl para reverter o cross-linking. Em seguida, as amostras foram incubadas a 45°C por 1h com o tampão pK (10 mM de EDTA, 40 mM de Tris-HCl pH 6,5 e 0,04 mg/mL de proteinase K) para remover proteínas por degradação. A extração das proteínas foi realizado com 1 volume de fenol/clorofórmio seguido de 1 volume de clorofórmio intercalando passos de centrifugação na rotação máxima por 10 minutos à TA. O DNA foi precipitado por 16 h com 1 µL de glicogênio 10 mg/mL, 0,1 volumes de NaAc 3 M e 2,5 volume de etanol absoluto. Após 20 minutos de centrifugação a TA e rotação máxima o precipitado foi lavado com etanol 70% e novamente centrifugado por 5 minutos a TA na rotação máxima. O sedimentado foi secado a TA e ressuspendido em 50 µL tampão TE. As sequências dos oligos utilizados nesta seção estão descritas na Tabela 5.

Tabela 5. Oligonucleotídeos utilizados para ChIP.

Nome[a]	Sequência
Cyp-F2	5´ CCCCACGATACCTCCACG 3´
Cyp-R3	5´ CTAAACACGGACTTCTGATTTGG 3´
PR5-F2	5´ GGTGCCAAACACCACCAC 3´
PR5-R3	5´ GGAAGGATGCATTTAATAAAGATAC 3´
CHS/Chip-F2	5´ CGATCCTCCCTGACTCTGAC 3´
CHS/Chip-R3	5´ ATCGAGTTCCAGTCGCTGAT 3´

[a]F e R: Iniciadores adiante e reverso, respectivamente.

RESULTADOS E DISCUSSÃO

Transativação de genes de citros por PthA *in vivo*

A fim de verificar se PthA é capaz de ligar as regiões promotoras previamente isoladas neste trabalho, as seguintes abordagens foram utilizadas: a imunoprecipitação de cromatina (*chromatin immunoprecipitation* ou ChIP), realizada no John Innes Centre em Norwich, no Reino Unido, em colaboração com o Dr. Robert Sablowski e a co-transformação de *N. tabacum*.

Para o ChIP, como controle endógeno de amplificação utilizou-se o gene chalcona sintáse (CHS) de *C. sinensis* que não é relacionado ao desenvolvimento dos sintomas de cancro. Iniciadores específicos para as regiões promotoras dos genes candidatos foram utilizados. As amplificações observadas para ambos '*input*' e o controle positivo garantem a eficiência da reação de PCR. As amplificações observadas para GUS (controle negativo) ocorre em função de uma pequena fração de DNA endógeno presente, normalmente esperado em experimentos de ChIP. Contudo é possível notar uma intensidade de amplificação maior para as amostras PthA2 e PthA4 tanto para *pr5* quanto para *cyp* quando comparadas como as amostras GUS (Figura 13). Embora os resultados indiquem a interação de PthA com as regiões promotoras em questão e sejam bastante promissores, são resultados preliminares e necessitam de validação por PCR quantitativo.

Para a co-transformação, a região promotora do *pr5* foi clonada de forma a dirigir a expressão do gene repórter GUS (pr5:GUS), que codifica para a enzima β-glucuronidase que, quando na presença do substrato X-Gluc, reage produzindo um pigmento azul. Como controles as construções 35S:GUS (GUS dirigido pelo promotor CaMV 35S) e *pr5*:GUS foram utilizadas. Os resultados obtidos até o momento indicam o PthA4 como a variante funcional de maior relevância, justificando a escolha desta variante para os ensaios. Neste sentido a co-transformação foi realizada com *pr5*:GUS + PthA4 e *pr5*:GUS + PthAΔRD (PthA sem o domínio interno, responsável pela atividade de ligação ao DNA).

A pigmentação azul é observada em abundância no controle 35S:GUS já que o promotor CaMV 35S é um promotor constitutivo forte (Figura 14A). Este resultado reflete a eficiência da transformação. Já para o controle *pr5*:GUS não foi observado pigmentação,

indicando que a região promotora não ativou a transcrição de GUS, indicando que este promotor, de citros, não é comumente expresso em tabaco (Figura 14B). Em contrapartida, o controle *pr5*:GUS na presença de PthA4 foi ativado, sugerindo que PthA4 é capaz de transativar o promotor *pr5* (Figura 14C). O controle pr5:GUS também parece ser ativado na presença de PthAΔRD, porem em nível inferior àquele observado na presença da proteína inteira (Figura 14D). Isto sugere que a atividade de ligação de DNA de PthA, de fato, reside no domínio interno o qual é essencial para transativação de genes *in planta*.

Embora estes resultados ainda sejam preliminares, em conjunto eles sugerem fortemente *pr5* como um alvo diretamente regulado por PthA4 durante a infecção por *X. citri*. Este é o primeiro trabalho a estudar PthA de um ponto de vista funcional, buscando a confirmação de atuação da referida proteína como fator de transcrição bem como seu alvo potenciais em citros.

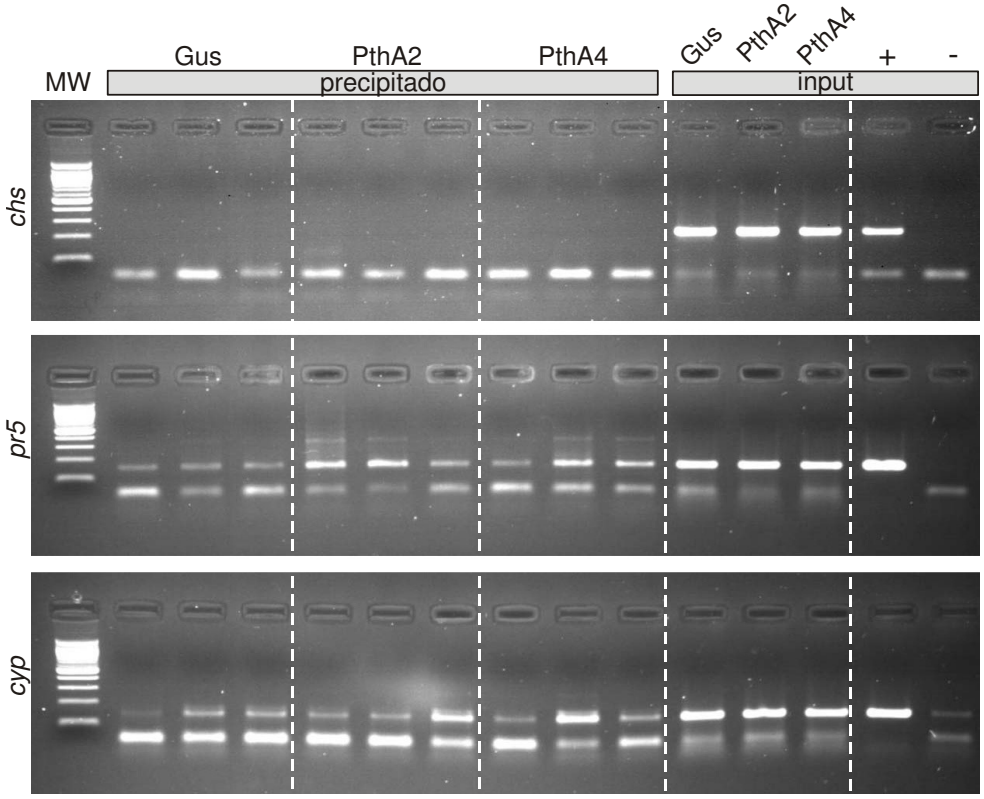


Figura 13. Imunoprecipitação de cromatina. Cromatina isolada a partir de tecidos de laranja doce com expressão transiente individual de *pthA2* ou *pthA4*. Como controle negativo tecido foi transformado com vetor vazio (Gus). '*input*': amostras coletadas antes da imunoprecipitação; MW: marcador de peso molecular; +: controle positivo de amplificação e −: controle negativo de amplificação.

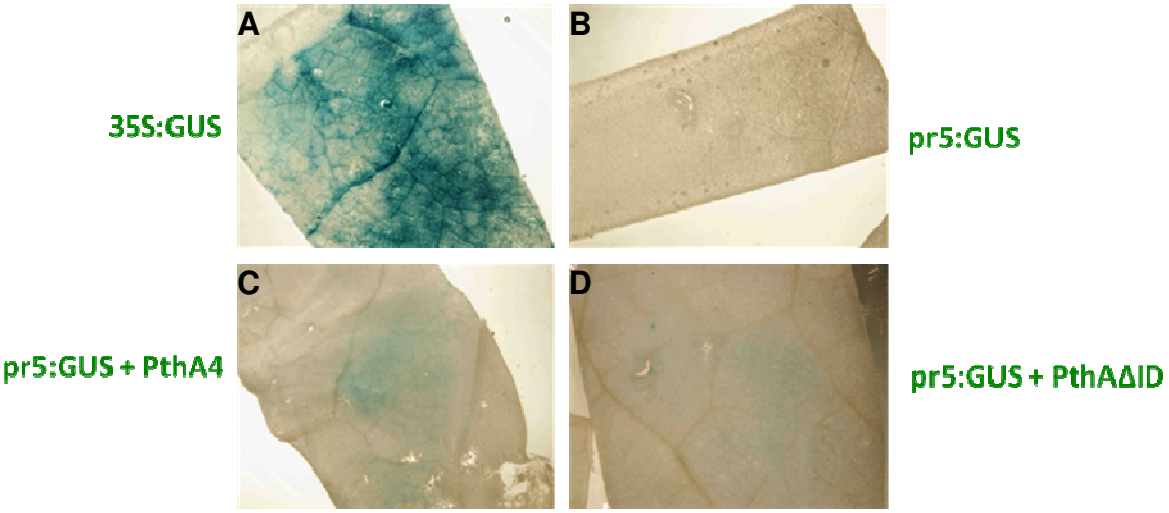


Figura 14. Experimento de co-transformação. Plantas de tabaco transformadas por Agro-infecção para testar a transativação do gene *pr5* por PthA4 *in planta*.

DISCUSSÃO GERAL

X. citri carrega proteínas que são essenciais para o desenvolvimento do cancro cítrico, dentre as quais PthA, um fator de patogenicidade, recebe destaque. A expressão transitória de PthA em laranja é capaz, por si só, de induzir hipertrofia e hiperplasia, processos biológicos que levam à cancrose (Duan *et al.*, 1999). Invariavelmente, este resultado nos remete à seguinte pergunta: por qual mecanismo molecular PthA opera? A princípio é preciso considerar: i) *X. citri* possui quatro variantes de PthA (PthA1-4), todas apresentando entre si diferenças mínimas que basicamente se concentram no domínio central da proteína, que possui atividade de ligação ao DNA; ii) PthA apresenta características típicas de fatores de transcrição de eucariotos e iii) exibe alta similaridade com AvrBs3, um efetor de *X. vesicatoria* que atua como fator de transcrição envolvido na reprogramação do transcriptoma do hospedeiro em benefício do patógeno. Até o momento, todas as vertentes sugerem a função de fator de transcrição para PthA.

A análise do perfil transcricional de laranja doce indica que *X. citri* modula a expressão de genes do hospedeiro para promover cancrose, visto que uma série de genes associados à divisão celular e remodelamento de parede celular foi observada com níveis de transcrição elevado na presença da bactéria (Cernadas *et al.*, 2008). Neste trabalho, descreve-se um perfil transcricional alterado em citros que independe do processo de síntese protéica, sugerindo assim a atividade sinérgica de proteínas efetoras de *X. citri* para modular a expressão de genes do hospedeiro. Desta forma, genes envolvidos em processos biológicos que poderiam contribuir para o desenvolvimento do cancro cítrico, bem como genes associados a respostas de defesa, foram identificados. Particularmente aqueles envolvidos em repostas de defesa foram selecionados para um estudo mais detalhado, dentre eles: *chitinase CHI1* (EST CX663308); *putative WRKY-type DNA binding protein* (EST CX046706); *cyclophilin* (CsCYP) (EST CX299605); *pathogenesis-related protein PR-1 precursor* (EST CF653559) e *pathogenesis-related protein pr5* (EST CK935296) (Tabela 1 do artigo submetido).

Ensaios de duplo-híbrido previamente realizados pelo nosso grupo identificaram a interação de PthA com uma ciclofilina, a mesma detectada nos microarranjos na presença

de ciclohexemida (Domingues *et al.*, 2010). As ciclofilinas são proteínas recrutadas para auxiliar o enovelamento de outras proteínas (Wang e Heitman, 2005). O papel das ciclofilinas no cancro cítrico não é entendido, no entanto, sabe-se que em alguns casos elas desempenham papel na interação planta-patógeno. É o caso da ciclofilina de *Arabidopsis* ROC1, que é responsável pela ativação de AvrRpt2, uma protease de *P. syringae* que induz HR em hospedeiros susceptíveis (Coaker *et al.*, 2005). Em citros o emprego de um inibidor de ciclofilina, a ciclosporina, infiltrado durante a infecção por *X. citri* atenuou o desenvolvimento do cancro cítrico (dados não publicados). Além disso, a ciclofilina foi encontrada associada a PthA2 em experimentos de co-imunoprecipitação (dados não publicados). Esses resultados sugerem uma participação efetiva da ciclofilina no processo de formação do cancro. Atualmente, a ciclofilina está sendo estudada mais detalhadamente por um dos membros do grupo.

Os genes que codificam as proteínas PR- 1 e 5 por sua vez também foram induzidas em resposta a expressão transiente de PthA (Tabela 2 do artigo submetido), mas não em resposta a expressão transiente de PthC (Tabela 3 do artigo submetido), sugerindo uma resposta induzida por ambos os PthA2 e 4. Contudo, é importante notar que algumas PRs também são induzidas por *X. aurantifolii* (Cernadas *et al.*, 2008), indicando que outro fator presente em *X. aurantifolii* que não os PthCs é capaz de induzir proteínas PR. Tanto PR-1 quanto PR-5 estão associadas a respostas de defesa a omicetos, enquanto as quitinases, que constituem as famílias PR-3, 4, 8 e 11 respondem a fungos (van Loon *et al.*, 2006). Em alguns casos as proteínas PRs podem conferir resistência ao hospedeiro. A super-expressão de *pr5* em plantas transgênicas de diferentes culturas promoveu resistência a certos tipos de patógenos, entre eles a laranja (Fagoaga *et al.*, 2001). Isso sugere que a ação destas proteínas é específica a determinados patógenos.

Os WRKY são fatores de transcrição que participam em diversos processos biológicos incluindo a ativação transcricional de genes de defesa (Eulgem e Somssich, 2007). Curiosamente, os WRKY são substratos de MAPKs (Andreasson *et al.*, 2005; Miao *et al.*, 2007). As MAPKs tem papel importante na regulação de diferentes processo de defesa e são rapidamente induzidas após o reconhecimento de algum patógeno (Ren *et al.*, 2006). Em geral, a cascata de sinalização por MAPKs é induzida logo nos primeiros

instantes da infecção (Cernadas *et al.*, 2008), o que torna relevante o estudo destas proteínas quanto a sua contribuição em respostas de defesa. A super-expressão destas proteínas em citros poderia significar resistência a *X. citri*, constituindo portanto uma estratégia plausível na geração de plantas resistentes à cancrose.

Embora o papel destas proteínas no cancro cítrico ainda permaneça obscuro, é interessante observar que em geral elas apresentam um elemento TATA-*box* em sua região promotora (Figura 11). De acordo com o código TAL, alguns promotores do hospedeiro são regulados especificamente pelos efetores TAL (Boch *et al.*, 2009; Moscou e Bogdanove, 2009). Recentemente, foi demonstrado que o efetor AvrBs3 se liga especificamente a um elemento TATA-*box*, mais tarde denominado *UPA-box* (Kay *et al.*, 2007; Römer *et al.*, 2007) que, curiosamente, é semelhante àqueles encontrados nos genes de citros regulados por *X. citri* (Figura 12). Através desta ligação AvrBs3 ativa um gene importante para desencadear sintomas de doença em tomate. Considerando a similaridade entre AvrBs3 e PthA, é possível especular a presença de sequências TATA-*box* distribuídas pelo genoma de citros, além daquelas aqui identificadas. Tais sequências funcionariam como elementos de regulação utilizados por efetores de *X. citri* para modular genes do hospedeiro.

Neste sentido a busca por prováveis sítios de ligação de efetores no genoma de citros seria bastante apropriada. O genoma completo de citros (*C. clementine* e *C. sinensis*) foi liberado recentemente possibilitando a busca por estas sequências a fim de verificar a presença de sítios de ligação para PthA com base no código TAL. Como ponto de partida, através de uma matriz desenvolvida neste trabalho, identificou-se nas regiões promotoras de genes positivamente regulados por PthA2 e 4 (Tabela 2 do artigo submetido), prováveis sítios de ligação que basicamente são sequências ricas em 'AT' (Figura 2B do artigo submetido). Frequentemente, essas regiões constituem TATA-*box*. Além do AvrBs3, outros efetores TAL, homólogos a PthA, também exibem afinidade por sequências ricas em 'AT', como por exemplo o AvrXa7 de *X. oryzae* (Yang *et al.*, 2000), reforçando a ideia de que PthA possua afinidade a sequências similares.

De fato, experimentos de EMSA demonstraram que tanto PthA2 quanto PthA4 possuem atividade de ligação ao DNA (Figura 3B do artigo submetido) e exibem afinidade

pelos TATA-*box* identificados neste estudo (Figura 3C do artigo submetido). Especialmente para *pr5* observou-se uma preferência de ligação ao TATA-*box* em relação ao UPA-*box* (Figura 3F do artigo submetido), reforçando a hipótese de que PthA funcione como fator de transcrição modulando a expressão deste gene. Apesar de preliminares, ensaios de co-imunoprecipitação de cromatina e co-transformação reforçam esta ideia, já que representam resultados *in vivo* que demonstram respectivamente a associação de PthA4 e *pr5 in planta* (Figura 13) e a ativação de um gene repórter dirigido pelo promotor de *pr5* (Figura 14). Pelo que sabemos, este é o primeiro trabalho que identifica genes de citros candidatos a modulação por um efetor de *X. citri*.

É interessante observar também que foram encontrados sítios de ligação para os PthCs 1 e 2, no entanto a regulação de genes em laranja foi negativa, sobretudo daqueles genes associados à sinalização por auxina e giberelina (Tabela 3 do artigo submetido). Em contrapartida, observamos a regulação positiva de auxina e giberelina por PthA (Tabela 2 do artigo submetido). Estes hormônios vegetais coordenam importantes processos biológicos que são necessários para estabelecer a interação planta-patógeno (Navarro *et al.*, 2006; Wang *et al.*, 2007; Zhang *et al.*, 2007; Domingo *et al.*, 2009). A auxina por exemplo, favorece o desenvolvimento de cancro cítrico e parece estabelecer um *cross-talk* com giberelina durante a cancrose, o que implica no envolvimento efetivo da giberelina durante o desenvolvimento do cancro cítrico (Cernadas e Benedetti, 2009). Assim, a ativação de genes de sinalização por auxina e giberelina parece ser uma etapa essencial para que *X. citri* possa induzir doença.

A expressão transiente de PthA em laranja resulta em um perfil de expressão gênica que sugere a modulação positiva de genes associados à divisão celular e remodelamento de parede celular, indicando a ativação dos processos de hipertrofia e hiperplasia. Em contrapartida a expressão transiente de PthC em laranja desencadeia uma modulação predominantemente negativa. É surpreendente notar que PthC2 regulou positivamente uma *S-adenosyl-L-homocysteine hydrolase*, que em *Arabidopsis* é codificada pelo gene *homology-dependent gene silencing* (*hog1*) (Tabela 3 do artigo submetido). *hog1* é necessário para o silenciamento de genes por metilação. Mutações deste gene levaram à remoção de metilação e elevaram os níveis transcricionais de *Arabidopsis* (Rocha *et al.*,

2005). Assim, a regulação positiva de *S-adenosyl-L-homocysteine hydrolase* pode explicar o perfil de expressão negativo induzido por PthC2.

Não se sabe por que razão *X. aurantifolii* não é capaz de induzir cancro cítrico em laranja, no entanto fica claro que uma reposta negativa é induzida pelos PthCs. De acordo com o modelo '*zigzag*', o reconhecimento de efetores por genes *R* pode induz uma resposta de defesa que resulta na imunidade do hospedeiro (Jones e Dangl, 2006). Por exemplo, em pimentão e tomate susceptíveis, a indução de HR deve-se à ativação do gene *bs3* por AvrBs3 (Römer *et al.*, 2009a). Assim, é possível que PthC ative genes *R* em laranja.

Aparentemente, PthA4 induziu um maior numero de genes associados ao desenvolvimento do cancro cítrico que PthA2, sobretudo aqueles envolvidos na sinalização por auxina e giberelina (Tabela 2 do artigo submetido). Este resultado corrobora com dados que apontam PthA4 como a variante funcional e essencial para patogenicidade de *X. citri* (Al-Saadi *et al.*, 2007). PthA4 é o equivalente de AvrBs3 em *X. citri*, com o arranjo de seu domínio interno organizado em 17,5 repetições. Isso sugere um mecanismo de ação semelhante. Contudo é bastante provável que outros PthA tenham papel aditivo contribuindo na modulação do transcriptoma do hospedeiro.

É ainda intrigante a presença de quatro variantes de PthA em *X. citri*. Ensaios de duplo híbrido demonstram a interação destas variantes com um número de proteínas de citros, além da capacidade de homodimerização (para todas as variantes) e heterodimerização (com exceção da heterodimerização entre PthA1 e 4) (Domingues *et al.*, 2010). Embora, até o momento, não se saiba se a transativação de genes ocorre com os efetores TAL na forma dimérica ou monomérica, pelo menos para o efetor AvrBs3 a dimerização é necessária para endereçá-lo ao núcleo (Gürlebeck *et al.*, 2005). Considerando que a ligação ocorra na forma dimérica, uma vez que o domínio interno de cada PthA sugere especificidade a uma sequência de DNA particular, a heterodimerização poderia conferir a capacidade de alternar entre alvos de ligação. A Figura 15 ilustra esta situação.

Por outro lado, experimentos com o RD2 demonstraram que este se encontra na forma monomérica, e sofre modificações conformacionais na presença de DNA (Murakami *et al.*, 2010). Este resultado sugere uma interação direta entre as moléculas. Além disso, o RD2 possui um domínio TPR formado majoritariamente por α-hélices que está relacionado

estruturalmente com o domínio PPR, que por sua vez apresenta um motivo de ligação de nucleotídeos (Williams-Carrier *et al.*, 2008; Pfalz *et al.*, 2009).

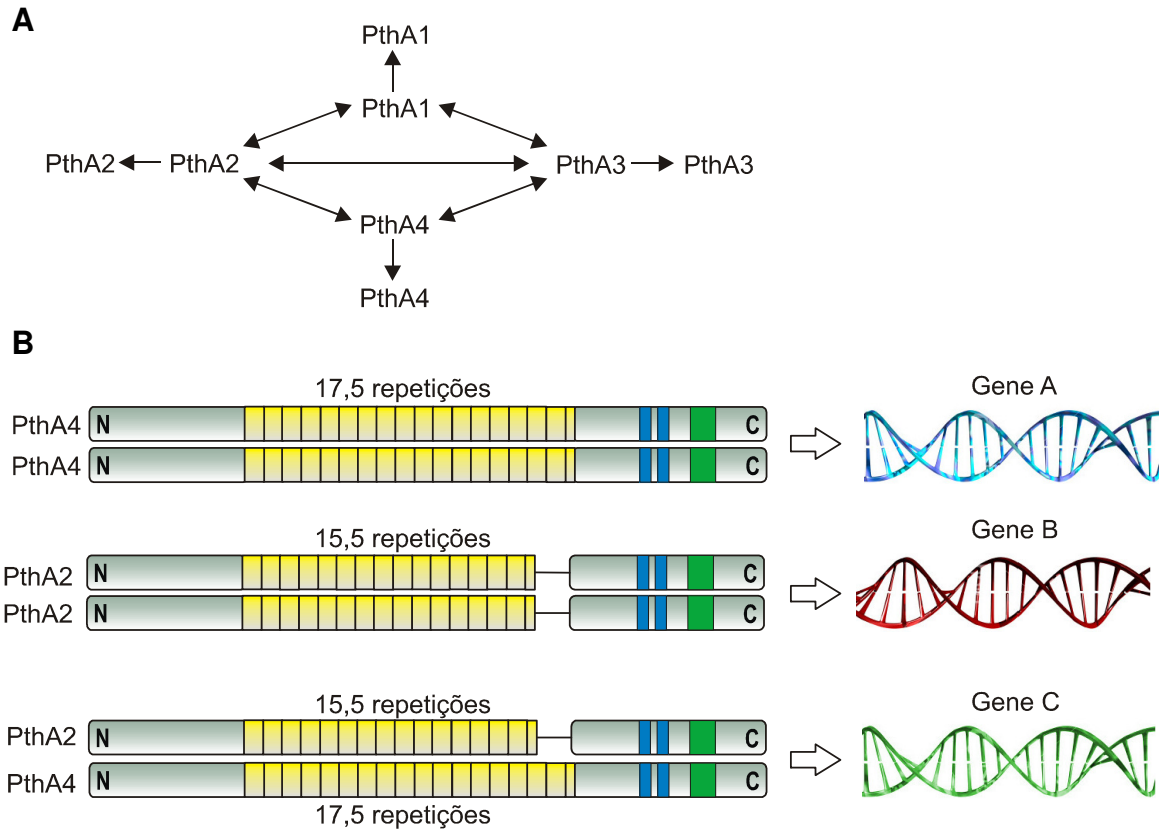


Figura 15. Modelo ilustrativo da dimerização de PthA. (**A**) Possibilidades de homo e heterodimerização. As setas indicam em que direção a dimerização pode ocorrer. (**B**) O homodímero de PthA4 liga o gene A enquanto o homodímero de PthA2 liga o gene B. A heterodimerização de PthA2/4 fornece a combinação de dois PthAs com diferentes domínios internos, isto poderia gerar uma proteína dimérica com especificidade de ligação a um terceiro alvo, neste caso o gene C. Em amarelo: domínio interno; em azul: domínios de localização nuclear; em verde: domínio ácido de ativação transcricional; N: N-terminal; C: C-terminal

A conformação monomérica pode ser explicada pela ausência do domínio LRR que PthA originalmente possui. Este domínio é importante para interação proteína-proteína e poderia promover a dimerização, no entanto não está presente no RD2 em função da estratégia de clonagem empregada para gerar a proteína recombinante. Apesar disso é

curiosa a interação de RD2 com DNA mesmo na forma monomérica. Esta é uma condição observada *in vitro* que ainda não é entendida, e também não observada *in vivo*. Ainda é possível que PthA sofra dimerização apenas para o transporte nuclear onde, posteriormente, poderia retornar a forma monômera para transativação de genes.

Mais interessante ainda, é a compactação de RD2, que corrobora com o código TAL, o qual prevê que cada dupla de resíduos de aminoácidos variáveis liga um único nucleotídeo (Boch e Bonas, 2010; Bogdanove *et al.*, 2010). Assim, o encurtamento da molécula representa uma espécie de ajuste que favorece a interação destes resíduos com as bases do DNA. Infelizmente, ainda não existe nenhuma estrutura 3D de efetores bacterianos depositada no PDB, sobretudo associados com DNA. Portanto, o mecanismo molecular exato de interação efetor-DNA permanece desconhecido. A maior dificuldade na cristalização de efetores TAL reside no seu tamanho. Apenas o domínio interno de alguns efetores TAL pode chegar até 33,5 repetições, cada uma com cerca 34 resíduos de aminoácidos (Boch e Bonas, 2010). Não obstante, alguns grupos de pesquisa já iniciaram a corrida pela busca da primeira estrutura.

O motivo pelo qual estão presentes quatro variantes de PthA em *X. citri* ainda é uma questão a ser respondida, entretanto, é possível que isso proporcione alguma vantagem na modulação do transcriptoma do hospedeiro. Até o momento, AvrBs3 é o único fator de transcrição efetivo de *X. vesicatoria* e ainda assim, AvrBs3 induz HR em hospedeiros susceptíveis e não hospedeiros. Enquanto isso *X. citri* é uma bactéria bastante agressiva que afeta praticamente todas as variedades de citros. Não se sabe se isso é devido à presença de PthA, contudo fica claro que o processo evolutivo favoreceu a conservação das quatro variantes. São quatro genes distintos que codificam os PthA1-4, que se encontram em plasmídeos (da Silva *et al.*, 2002; Brunings e Gabriel, 2003), e é improvável que durante o processo evolutivo *X. citri* tenha mantido estes genes sem necessidade.

Finalmente, a possibilidade de identificar *in silico* potenciais sequências alvos de PthA juntamente com o conjunto de promotores de citros isolados neste trabalho, contribui para posteriores estudos de regulação efetiva de genes de citros bem como o efeito de tais genes para o desenvolvimento dos sintomas do cancro cítrico. Alternativamente, determinados promotores também podem ser úteis na geração de plantas resistentes a *X.*

citri. Uma estratégia atualmente abordada pelo nosso grupo, que se encontra em andamento, consiste na clonagem de promotores que são especificamente ativados na presença da bactéria para ativar genes de defesa (dados não publicados).

CONCLUSÃO

Durante a patogênese, a bactéria *X. citri*, causadora do cancro cítrico, utiliza uma série de proteínas efetores que são essenciais para o desenvolvimento da cancrose porém, o mecanismo pelo qual essas proteínas operam são escassos. Neste sentido, o presente estudo buscou fornecer informações experimentais que possam contribuir para o entendimento a nível molecular do mecanismo de ação da proteína efetora PthA.

Através de uma série de microarranjos foi possível identificar um perfil de expressão gênica em citros dependente de PthA. Genes intimamente relacionados com hipertrofia e hiperplasia, processos que levam à cancrose, foram identificados com altos níveis de expressão. Em especial, a expressão transiente de *pthA4* em laranja doce resultou na indução de um número de genes relacionados principalmente a divisão e aumento de volume celular, alem daqueles relacionados à síntese e remodelamento de parede celular. A expressão de alguns desses genes foi identificada tanto no tratamento com ciclohexemida como nos tecidos com expressão transiente de *pthA*. Os genes aparentemente insensíveis a ciclohexemida, em potencial são induzidos diretamente por PthA.

Dentre vários genes previamente selecionados, foi possível isolar com sucesso as regiões promotoras de cinco deles possivelmente envolvidos em resposta de defesa ou processos de desenvolvimento de cancro cítrico. Desses destacamos o gene *pr5*. As análises *in silico* das regiões promotoras revelaram a presença de elementos típicos de regulação transcricional tais como TATA-*box*. Curiosamente os TATA-*box* preditos são bastante semelhantes ao *UPA-box*, o qual é regulado por AvrBs3. A alta similaridade entre *UPA-box* e o TATA-*box* predito na região promotora do *pr5* encorajou a realização experimentos para verificar a interação proteína-DNA. Nossos resultados sugerem afinidade da proteína pela região do TATA-*box* predita para o *pr5*, sobretudo para PthA4, além de confirmar a interação via domínio central. Ambos os experimentos de ChIP e co-transformação, embora com resultados ainda preliminares, sugerem a ligação tanto de *pr5* quanto de *cyp in planta* por ambos os PthA2 e 4, e a transativação direta de *pr5* por PthA4 em tabaco, respectivamente.

Em síntese nossos resultados representam os primeiros dados experimentais que demonstram a afinidade de PthA por sequências específicas de genes de citros associados aos sintomas de hipertrofia e hiperplasia tecidual, que naturalmente originam o cancro cítrico, reforçando assim, a hipótese de PthA como um fator de transcrição.

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Uso exclusivo da CIBio:

Número de projeto / processo: CEBD4.04

Formulário de encaminhamento de projetos de pesquisa para análise pela CIBio - Comissão Interna de Biossegurança da ABTLuS – Associação Brasileira de Tecnologia de Luz Síncrotron

Título do projeto: Identificação de genes de *Citrus sinensis* com expressão dependente da proteína PthA de *Xanthomonas axonopodis citri* e isolamento de elementos *cis*-regulatórios ligantes de PthA.

Pesquisador responsável: Celso Eduardo Benedetti

Experimentador: André Luiz Araújo Pereira

Nível do treinamento do experimentador: [ ]-Iniciação científica, [ ]-mestrado, [x]-doutorado, [ ]-doutorado direto, [ ]-pós-doutorado, [ ]-nível técnico, [ ]-outro, especifique:_____

Resumo do projeto:

O cancro cítrico, doença causada pela bactéria *Xanthomonas axonopodis* pv. *citri* (Xac) se caracteriza pela formação de pústulas na superfície de folhas, frutos e ramos. Dados recentes mostram que proteínas efetoras da bactéria são transferidas para o interior das células da planta hospedeira, resultando na hiperplasia e hipertrofia do tecido. Entre as proteínas efetoras de Xac, PthA é a mais estudada. Neste sentido, este trabalho visa o estudo da interação da bactéria Xac e citros a nível molecular buscando em especial o entendimento do mecanismo de ação da proteína PthA como fator de transcrição e a identificação dos genes alvos na planta. Através da técnica chamada Systematic Evolution of Ligands by EXponential enrichment (SELEX) serão selecionadas potenciais seqüências de DNA alvo ligadas pela proteína PthA, as quais serão submetidas à gel-shift para confirmação da interação. A análise de vários fragmentos de DNA ligadores de PthA poderá revelar uma seqüência consenso que seria utilizada na identificação de promotores de citros alvos de PthA. O projeto prevê ainda a análise por microarray de protoplastos de citros transformados com o gene pthA ou proteína PthA recombinante a afim de revelar genes de laranja cuja expressão é dependente de PthA. Uma vez identificados tais genes, seus promotores serão testados através de gel-shift para verificar interação física com PthA. A identificação de promotores alvos da PthA permitirá não somente o melhor entendimento da função dessa família de proteínas Avr, mas também possibilitará o desenvolvimento de estratégias moleculares para inibição dessa proteína como possível alternativa para o combate ao cancro cítrico.

A CIBio analisou este projeto em reunião realizada no dia: 28/11/2008.

Parecer final: [X]-projeto aprovado, [ ]-projeto recusado, [ ]-projeto com deficiências, favor comentários abaixo:

_____

_____

_____

_____

Presidente de CIBio - ABTLuS
Prof. Dr. Jörg Kobarg

Membro da CIBio - ABTLuS
Prof. Dr. Celso Eduardo Benedetti

Membro da CIBio - ABTLuS
Prof. Dr. Nilson Ivo Tonin Zanchin

– ANEXO I –

Conjunto de tabelas suplementares referentes ao artigo científico submetido que constam do capítulo I.

GUS Vs PthA2, fold change > 3x

Auxin mobilization and signaling

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.26243.1.S1_at	3,17	up	CN184032	NP_194456.1	1E-41	auxin-responsive GH3 family protein [Arabidopsis thaliana]
Cit.35736.1.S1_s_at	3,19	up	CK937473	Q9ZRA4	9E-76	Auxin-binding protein ABP19a precursor
Cit.31860.1.S1_s_at	8,26	up	AU300809	S33621	5E-13	ADR11-2 protein - soybean (fragment) emb|CAA49341.1| ADR11 [Glycine max]

Carbohydrate, Aminoacid and Nucleotide Metabolism

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.28009.1.S1_s_at	3,14	up	CX671808	NP_173510.1	1E-55	phosphate transporter family protein [Arabidopsis thaliana]
Cit.28009.1.S1_at	3,31	up	CX671808	NP_173510.1	1E-55	phosphate transporter family protein [Arabidopsis thaliana]
Cit.5700.1.S1_at	4,12	up	CX050506	AAM61517.1	1E-112	uracil-DNA glycosylase, putative [Arabidopsis thaliana]

Cell division and morphogenesis

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.30902.1.S1_at	3,02	up	DN798551	AAB50686.1	5E-14	Con1 [Homo sapiens]
Cit.4118.1.S1_s_at	3,18	up	CK932935	CAE02924.1	2E-69	OSJNBb0108J11.17 [Oryza sativa (japonica cultivar-group)]
Cit.19872.1.S1_s_at	3,20	up	CK934140	NP_197027.1	1E-38	gibberellin-regulated protein 4 (GASA4) / gibberellin-responsive protein 4 [Arabidopsis thaliana]
Cit.3665.1.S1_s_at	3,34	up	CF508354	CAB45241.1	7E-34	GEG protein [Gerbera hybrid cultivar]
Cit.12502.1.S1_s_at	3,61	up	CX050861	AAT39315.1	2E-90	putative senescence-associated protein [Solanum demissum]
Cit.5300.1.S1_at	3,87	up	CV713508	AAL34163.1	5E-70	putative dUTP pyrophosphatase [Arabidopsis thaliana]
Cit.12503.1.S1_at	4,46	up	DN135183	NP_190146.1	3E-82	senescence-associated family protein [Arabidopsis thaliana]
Cit.11057.1.S1_s_at	4,93	up	CV716755	AAT40548.1	3E-83	putative vicilin [Solanum demissum]
Cit.29007.1.S1_at	5,32	up	CX637545	S71371	4E-35	gibberellin-regulated protein GASA5 precursor [Arabidopsis thaliana]
Cit.3665.1.S1_at	5,46	up	CF508354	CAB45241.1	7E-34	GEG protein [Gerbera hybrid cultivar]
Cit.18235.1.S1_at	7,83	up	CK936113	NP_187298.1	2E-92	GNS1/SUR4 membrane family protein [Arabidopsis thaliana]

Cell-wall synthesis and remodelling

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.1385.1.S1_s_at	3,03	up	CV887009	T10737	7E-67	extensin-like cell wall protein - sea-island cotton
Cit.14266.1.S1_at	3,04	up	CX666282	CAB88664.1	1E-164	putative glucosyltransferase [Cicer arietinum]
Cit.21654.1.S1_s_at	3,11	up	CX299958	AAC35981.1	5E-27	chitinase CHI1 [Citrus sinensis]
Cit.399.1.S1_s_at	3,12	up	CF837795	AAR09170.1	2E-45	alpha-expansin 3 [Populus tremula x Populus tremuloides]
Cit.31337.1.S1_at	3,13	up	CF505722	CAA10382.2	7E-92	alpha-D-xylosidase [Tropaeolum majus]
Cit.10594.1.S1_at	3,28	up	DN624837	AAC35981.1	1E-78	chitinase CHI1 [Citrus sinensis]
Cit.14522.1.S1_at	3,29	up	CX043829	CAA10382.2	1E-82	alpha-D-xylosidase [Tropaeolum majus]
Cit.11898.1.S1_at	3,39	up	CD573639	AAL57631.1	4E-69	At1g78060/F28K19_32 [Arabidopsis thaliana]
Cit.14913.1.S1_s_at	3,41	up	CX043703	CAA77656.1	6E-73	acidic chitinase III [Nicotiana tabacum]
Cit.18089.1.S1_x_at	3,46	up	CK740200	T06782	2E-67	extensin - soybean
Cit.23967.1.S1_s_at	3,48	up	CV710106	CAA10382.2	4E-60	alpha-D-xylosidase [Tropaeolum majus]
Cit.2701.1.S1_at	3,61	up	CV710968	AAO92753.1	2E-56	arabinogalactan protein [Gossypium hirsutum]
Cit.2701.1.S1_at	3,61	up	CV710968	AAO92753.1	2E-56	arabinogalactan protein [Gossypium hirsutum]
Cit.3283.1.S1_s_at	3,72	up	DN619806	BAB59066.1	1E-148	pectate lyase [Salix gilgiana]
Cit.30534.1.S1_s_at	3,79	up	CK934786	S65063	4E-21	fiber protein E6 (clones SIE6-2A and SIE6-3B) - sea-island cotton
Cit.9706.1.S1_s_at	3,79	up	CV886686	CAA03908.1	1E-145	beta-1,3-glucanase [Citrus sinensis]
Cit.18089.1.S1_at	3,89	up	CK740200	T06782	2E-67	extensin - soybean
Cit.1386.1.S1_at	3,99	up	CX672178	T10737	5E-75	extensin-like cell wall protein - sea-island cotton
Cit.7877.1.S1_at	4,16	up	CX667721	S48032	5E-95	cim1 protein - soybean
Cit.19350.1.S1_s_at	4,24	up	CK934803	NP_196320.1	4E-42	aspartyl protease family protein [Arabidopsis thaliana]
Cit.13410.1.S1_s_at	4,38	up	CX669534	AAP04044.1	1E-103	putative pectin methylesterase [Arabidopsis thaliana]
Cit.2700.1.S1_s_at	4,40	up	CV709336	AAO92753.1	7E-81	arabinogalactan protein [Gossypium hirsutum]
Cit.2700.1.S1_s_at	4,40	up	CV709336	AAO92753.1	7E-81	arabinogalactan protein [Gossypium hirsutum]
Cit.6334.1.S1_at	4,46	up	CV715984	BAB10434.1	5E-99	ABC transporter-like protein [Arabidopsis thaliana]
Cit.2945.1.S1_s_at	4,49	up	CV887291	CAC94006.1	1E-148	endo-beta-1,4-glucanase [Fragaria x ananassa]
Cit.4516.1.S1_s_at	4,59	up	CV886618	AAP33475.1	1E-116	polygalacturonase-like protein [Fragaria x ananassa]
Cit.15242.1.S1_at	4,68	up	CX292066	AAC35981.1	1E-101	chitinase CHI1 [Citrus sinensis]
Cit.1077.1.S1_s_at	4,77	up	CF829440	AAQ84042.1	1E-93	pectate lyase [Malus x domestica]
Cit.17888.1.S1_s_at	4,89	up	CF838857	NP_177929.1	1E-106	glycosyl hydrolase family 3 protein [Arabidopsis thaliana]
Cit.5084.1.S1_at	5,04	up	CN186015	AAQ84323.1	2E-30	fiber protein Fb31 [Gossypium barbadense]
Cit.311.1.S1_s_at	5,47	up	CN182741	BAB16431.1	8E-18	P-rich protein NtEIG-C29 [Nicotiana tabacum]
Cit.20158.1.S1_x_at	5,89	up	CF834901	NP_191008.1	7E-23	aspartyl protease family protein [Arabidopsis thaliana]
Cit.12490.1.S1_s_at	6,13	up	CX044619	AAM20001.1	1E-134	putative polygalacturonase PG1 [Arabidopsis thaliana]
Cit.20071.1.S1_s_at	6,58	up	CX666732	AAP33475.1	4E-90	polygalacturonase-like protein [Fragaria x ananassa]

GUS Vs PthA2, fold change > 3x

Auxin mobilization and signaling

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
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Cit.35736.1.S1_s_at	3,19	up	CK937473	Q9ZRA4	9E-76	Auxin-binding protein ABP19a precursor
Cit.31860.1.S1_s_at	8,26	up	AU300809	S33621	5E-13	ADR11-2 protein - soybean (fragment) emb|CAA49341.1| ADR11 [Glycine max]

Carbohydrate, Aminoacid and Nucleotide Metabolism

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.28009.1.S1_s_at	3,14	up	CX671808	NP_173510.1	1E-55	phosphate transporter family protein [Arabidopsis thaliana]
Cit.28009.1.S1_at	3,31	up	CX671808	NP_173510.1	1E-55	phosphate transporter family protein [Arabidopsis thaliana]
Cit.5700.1.S1_at	4,12	up	CX050506	AAM61517.1	1E-112	uracil-DNA glycosylase, putative [Arabidopsis thaliana]

Cell division and morphogenesis

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
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Cit.19872.1.S1_s_at	3,20	up	CK934140	NP_197027.1	1E-38	gibberellin-regulated protein 4 (GASA4) / gibberellin-responsive protein 4 [Arabidopsis thaliana]
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Cit.12502.1.S1_s_at	3,61	up	CX050861	AAT39315.1	2E-90	putative senescence-associated protein [Solanum demissum]
Cit.5300.1.S1_at	3,87	up	CV713508	AAL34163.1	5E-70	putative dUTP pyrophosphatase [Arabidopsis thaliana]
Cit.12503.1.S1_at	4,46	up	DN135183	NP_190146.1	3E-82	senescence-associated family protein [Arabidopsis thaliana]
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Cit.29007.1.S1_at	5,32	up	CX637545	S71371	4E-35	gibberellin-regulated protein GASA5 precursor [Arabidopsis thaliana]
Cit.3665.1.S1_at	5,46	up	CF508354	CAB45241.1	7E-34	GEG protein [Gerbera hybrid cultivar]
Cit.18235.1.S1_at	7,83	up	CK936113	NP_187298.1	2E-92	GNS1/SUR4 membrane family protein [Arabidopsis thaliana]

Cell-wall synthesis and remodelling

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
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Cit.14266.1.S1_at	3,04	up	CX666282	CAB88664.1	1E-164	putative glucosyltransferase [Cicer arietinum]
Cit.21654.1.S1_s_at	3,11	up	CX299958	AAC35981.1	5E-27	chitinase CHI1 [Citrus sinensis]
Cit.399.1.S1_s_at	3,12	up	CF837795	AAR09170.1	2E-45	alpha-expansin 3 [Populus tremula x Populus tremuloides]
Cit.31337.1.S1_at	3,13	up	CF505722	CAA10382.2	7E-92	alpha-D-xylosidase [Tropaeolum majus]
Cit.10594.1.S1_at	3,28	up	DN624837	AAC35981.1	1E-78	chitinase CHI1 [Citrus sinensis]
Cit.14522.1.S1_at	3,29	up	CX043829	CAA10382.2	1E-82	alpha-D-xylosidase [Tropaeolum majus]
Cit.11898.1.S1_at	3,39	up	CD573639	AAL57631.1	4E-69	At1g78060/F28K19_32 [Arabidopsis thaliana]
Cit.14913.1.S1_s_at	3,41	up	CX043703	CAA77656.1	6E-73	acidic chitinase III [Nicotiana tabacum]
Cit.18089.1.S1_x_at	3,46	up	CK740200	T06782	2E-67	extensin - soybean
Cit.23967.1.S1_s_at	3,48	up	CV710106	CAA10382.2	4E-60	alpha-D-xylosidase [Tropaeolum majus]
Cit.2701.1.S1_at	3,61	up	CV710968	AAO92753.1	2E-56	arabinogalactan protein [Gossypium hirsutum]
Cit.2701.1.S1_at	3,61	up	CV710968	AAO92753.1	2E-56	arabinogalactan protein [Gossypium hirsutum]
Cit.3283.1.S1_s_at	3,72	up	DN619806	BAB59066.1	1E-148	pectate lyase [Salix gilgiana]
Cit.30534.1.S1_s_at	3,79	up	CK934786	S65063	4E-21	fiber protein E6 (clones SIE6-2A and SIE6-3B) - sea-island cotton
Cit.9706.1.S1_s_at	3,79	up	CV886686	CAA03908.1	1E-145	beta-1,3-glucanase [Citrus sinensis]
Cit.18089.1.S1_at	3,89	up	CK740200	T06782	2E-67	extensin - soybean
Cit.1386.1.S1_at	3,99	up	CX672178	T10737	5E-75	extensin-like cell wall protein - sea-island cotton
Cit.7877.1.S1_at	4,16	up	CX667721	S48032	5E-95	cim1 protein - soybean
Cit.19350.1.S1_s_at	4,24	up	CK934803	NP_196320.1	4E-42	aspartyl protease family protein [Arabidopsis thaliana]
Cit.13410.1.S1_s_at	4,38	up	CX669534	AAP04044.1	1E-103	putative pectin methylesterase [Arabidopsis thaliana]
Cit.2700.1.S1_s_at	4,40	up	CV709336	AAO92753.1	7E-81	arabinogalactan protein [Gossypium hirsutum]
Cit.2700.1.S1_s_at	4,40	up	CV709336	AAO92753.1	7E-81	arabinogalactan protein [Gossypium hirsutum]
Cit.6334.1.S1_at	4,46	up	CV715984	BAB10434.1	5E-99	ABC transporter-like protein [Arabidopsis thaliana]
Cit.2945.1.S1_s_at	4,49	up	CV887291	CAC94006.1	1E-148	endo-beta-1,4-glucanase [Fragaria x ananassa]
Cit.4516.1.S1_s_at	4,59	up	CV886618	AAP33475.1	1E-116	polygalacturonase-like protein [Fragaria x ananassa]
Cit.15242.1.S1_at	4,68	up	CX292066	AAC35981.1	1E-101	chitinase CHI1 [Citrus sinensis]
Cit.1077.1.S1_s_at	4,77	up	CF829440	AAQ84042.1	1E-93	pectate lyase [Malus x domestica]
Cit.17888.1.S1_s_at	4,89	up	CF838857	NP_177929.1	1E-106	glycosyl hydrolase family 3 protein [Arabidopsis thaliana]
Cit.5084.1.S1_at	5,04	up	CN186015	AAQ84323.1	2E-30	fiber protein Fb31 [Gossypium barbadense]
Cit.311.1.S1_s_at	5,47	up	CN182741	BAB16431.1	8E-18	P-rich protein NtEIG-C29 [Nicotiana tabacum]
Cit.20158.1.S1_x_at	5,89	up	CF834901	NP_191008.1	7E-23	aspartyl protease family protein [Arabidopsis thaliana]
Cit.12490.1.S1_s_at	6,13	up	CX044619	AAM20001.1	1E-134	putative polygalacturonase PG1 [Arabidopsis thaliana]
Cit.20071.1.S1_s_at	6,58	up	CX666732	AAP33475.1	4E-90	polygalacturonase-like protein [Fragaria x ananassa]

Cit.3554.1.S1_s_at	6,83	up	CX663293	T07885	1E-124	cellulase (EC 3.2.1.4) - sweet orange gb|AAB65156.1| basic cellulase [C. sinensis]
Cit.11915.1.S1_s_at	6,92	up	CX071344	AAC17605.1	6E-45	Contains similarity to proline-rich protein, gb|S68113 from Brassica napus. [Arabidopsis thalia
Cit.17853.1.S1_s_at	7,42	up	CK933446	NP_563715.1	1E-145	pectate lyase family protein [Arabidopsis thaliana]
Cit.11685.1.S1_s_at	8,00	up	CX663825	AAM65121.1	5E-74	putative proline-rich cell wall protein [Arabidopsis thaliana]
Cit.10437.1.S1_s_at	8,86	up	CX665316	AAP33475.1	1E-144	polygalacturonase-like protein [Fragaria x ananassa]
Cit.4483.1.S1_s_at	8,94	up	CX077158	BAB11424.1	5E-79	beta-xylosidase [Arabidopsis thaliana]
Cit.26012.1.S1_at	9,44	up	CF833607	AAF35897.1	8E-56	pectin methylesterase isoform alpha [Vigna radiata]
Cit.21559.1.S1_s_at	10,21	up	CX051882	AAP33475.1	4E-33	polygalacturonase-like protein [Fragaria x ananassa]
Cit.2949.1.S1_s_at	10,91	up	CN182557	T10523	1E-153	xyloglucan endo-1,4-beta-D-glucanase (EC 3.2.1.-) 1 - common nasturtium
Cit.25554.1.S1_at	12,83	up	CX669376	AAK50769.1	1E-63	polygalacturonase [Pisum sativum]
Cit.3390.1.S1_at	13,91	up	CV709535	NP_197666.1	1E-128	glycosyl transferase family 2 protein [Arabidopsis thaliana]
Cit.35754.1.S1_at	23,28	up	CB250305	NP_191544.1	2E-09	polygalacturonase, putative [Arabidopsis thaliana]
Cit.35756.1.S1_at	26,74	up	CB250319	AAC64184.1	2E-96	endopolygalacturonase [Prunus persica]
Cit.32767.1.S1_at	35,41	up	CX294670	AAP21999.1	1E-58	endopolygalacturonase [Prunus persica]
Cit.2392.1.S1_at	81,46	up	CF831790	AAB65155.1	0.0	acidic cellulase [Citrus sinensis]

Disease resistance, defence and stress response

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.17919.1.S1_s_at	3,03	up	DN795283	Q93ZH0	1E-106	LysM-domain GPI-anchored protein 1 precursor
Cit.18517.1.S1_s_at	3,07	up	CX293755	AAO27256.1	4E-39	putative NADH-dehydrogenase [Pisum sativum]
Cit.9584.1.S1_x_at	3,13	up	DR403286	AAG30140.1	9E-83	glutathione S-transferase [Arabidopsis thaliana]
Cit.10661.1.S1_at	3,13	up	CB291294	BAB82419.1	0.0	acid invertase [Citrus unshiu]
Cit.5228.1.S1_s_at	3,18	up	DN622543	AAK59558.1	1E-31	putative receptor-protein kinase [Arabidopsis thaliana]
Cit.275.1.S1_s_at	3,23	up	CF833152	NP_188923.1	4E-53	chlorophyll A-B binding family protein / early light-induced protein (ELIP) [Arabidopsis thaliana
Cit.21128.1.S1_at	3,28	up	DN799150	BAB02603.1	4E-27	leucoanthocyanidin dioxygenase-like protein [Arabidopsis thaliana]
Cit.4457.1.S1_s_at	3,28	up	CD574780	AAP03023.1	3E-82	acyl-activating enzyme 12 [Arabidopsis thaliana]
Cit.14757.1.S1_at	3,30	up	CK702467	NP_566082.1	6E-37	calcium-binding protein, putative [Arabidopsis thaliana]
Cit.9301.1.S1_s_at	3,31	up	CN190145	BAD11070.1	1E-102	HSR203J like protein [Capsicum chinense]
Cit.2113.1.S1_at	3,31	up	CK934775	NP_176113.1	2E-59	disease resistance-responsive protein-related / dirigent protein-related [Arabidopsis thaliana]
Cit.5956.1.S1_at	3,31	up	CV885460	AAM18791.1	8E-31	immuno-reactant natriuretic peptide-like protein [Erucastrum strigosum]
Cit.19313.1.S1_s_at	3,35	up	CX308038	EAL71975.1	2E-04	putative protein serine/threonine kinase [Dictyostelium discoideum]
Cit.8908.1.S1_s_at	3,36	up	CX674698	AAC39480.1	3E-92	aquaporin [Vernicia fordii]
Cit.11230.1.S1_s_at	3,37	up	CK936665	BAB16426.1	4E-78	elicitor inducible gene product EIG-I24 [Nicotiana tabacum]
Cit.11691.1.S1_at	3,50	up	CF832883	CAB42906.1	3E-48	calmodulin-like protein [Arabidopsis thaliana]
Cit.106.1.S1_s_at	3,67	up	CF835209	AAM62745.1	1E-116	nucleoid DNA-binding-like protein [Arabidopsis thaliana]
Cit.2027.1.S1_s_at	3,70	up	DN617689	AAD02832.1	1E-55	raffinose synthase [Cucumis sativus]
Cit.165.1.S1_s_at	3,71	up	CB291284	AAO33591.1	1E-55	putative early light induced protein [Arachis hypogaea]
Cit.29369.1.S1_x_at	3,72	up	DR405740	Q39967	1E-18	Major latex allergen Hev b 5
Cit.32625.1.S1_s_at	3,78	up	CX292843	CAA58733.1	4E-07	PAR-1a [Nicotiana tabacum]
Cit.26982.1.S1_at	3,80	up	CV716390	NP_189066.1	1E-92	leucine-rich repeat transmembrane protein kinase, putative [Arabidopsis thaliana]
Cit.8282.1.S1_s_at	3,81	up	CX303177	AAD11482.1	1E-106	peroxidase precursor [Glycine max]
Cit.23259.1.S1_s_at	3,83	up	CX676279	AAB95118.1	3E-88	pathogenesis-related group 5 protein [Brassica rapa]
Cit.19727.1.S1_x_at	3,84	up	CK937930	BAB40143.1	4E-31	plasma membrane intrinsic protein 2-2 [Pyrus communis]
Cit.19769.1.S1_x_at	3,86	up	CK937268	AAO33591.1	4E-58	putative early light induced protein [Arachis hypogaea]
Cit.10864.1.S1_s_at	3,87	up	CV706080	CAE76632.1	3E-89	leucine rich repeat protein [Cicer arietinum]
Cit.1820.1.S1_at	3,94	up	DN797852	BAA21921.1	9E-37	ZPT2-12 [Petunia x hybrida]
Cit.10235.1.S1_at	4,06	up	CN182037	S62698	2E-64	photoassimilate-responsive protein precursor (clone PAR-1a) - common tobacco
Cit.31825.1.S1_at	4,25	up	AU300664	NP_194308.1	6E-30	pathogenesis-related protein, putative [Arabidopsis thaliana]
Cit.16900.1.S1_x_at	4,37	up	CB610530	Q39967	6E-21	Major latex allergen Hev b 5
Cit.40341.1.S1_x_at	4,44	up	DR405740	Q39967	1E-20	Major latex allergen Hev b 5
Cit.376.1.S1_x_at	4,58	up	CX666561	Q39967	5E-22	Major latex allergen Hev b 5
Cit.100.1.S1_x_at	4,70	up	CK934397	Q39967	3E-21	Major latex allergen Hev b 5
Cit.18517.1.S1_at	4,71	up	CX293755	AAO27256.1	4E-39	putative NADH-dehydrogenase [Pisum sativum]
Cit.4661.1.S1_s_at	4,72	up	CV713066	AAT68601.1	1E-118	benzoyl coenzyme A: benzyl alcohol benzoyl transferase [Petunia x hybrida]
Cit.31377.1.S1_at	5,15	up	DN619712	AAB81668.1	8E-81	NAM (no apical meristem)-like protein [Arabidopsis thaliana]
Cit.2848.1.S1_s_at	5,20	up	DN795261	NP_188718.1	1E-97	leucine-rich repeat family protein [Arabidopsis thaliana]

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Auxin mobilization and signaling

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.31860.1.S1_s_at	7,19	up	AU300809	S33621	5E-13	ADR11-2 protein - soybean (fragment) emb|CAA49341.1| ADR11 [Glycine max]
Cit.5583.1.S1_at	4,55	up	CV885552	T50834	1E-153	anthranilate synthase (EC 4.1.3.27) alpha chain [imported] -
Cit.23340.1.S1_at	4,03	up	CX053885	AAM55306.1	3E-91	auxin influx carrier protein [Medicago truncatula]
Cit.15048.1.S1_s_at	3,63	up	CV886883	BAD19065.1	6E-68	auxin response factor 5 [Cucumis sativus]
Cit.35736.1.S1_s_at	5,23	up	CK937473	Q9ZRA4	9E-76	Auxin-binding protein ABP19a precursor gb|AAD00295.1| auxin-binding protein ABP19
Cit.35241.1.S1_at	3,07	up	CK936210	AAL08561.1	8E-15	auxin-regulated protein [Lycopersicon esculentum]
Cit.27799.1.S1_at	5,90	up	CX675673	XP_483243.1	2E-34	auxin-regulated protein-like [Oryza sativa (japonica cultivar-group)]
Cit.3767.1.S1_at	4,75	up	CV706455	CAC12996.1	1E-167	putative AUX1-like permease [Medicago truncatula]
Cit.3173.1.S1_s_at	3,50	up	CF834975	BAC75413.1	9E-06	putative auxin-induced protein [Oryza sativa (japonica cultivar-group)]
Cit.36707.1.S1_at	3,06	up	CV704545	BAB10403.1	1E-77	unnamed protein product [Arabidopsis thaliana]
Cit.25125.1.S1_at	3,32	up	CX307176	BAB10403.1	1E-33	unnamed protein product [Arabidopsis thaliana]

Carbohydrate, Aminoacid and Nucleotide Metabolism

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.4941.1.S1_s_at	3,01	up	CV714280	NP_191610.3	8E-94	enoyl-CoA hydratase/isomerase family protein [Arabidopsis thaliana]
Cit.3923.1.S1_s_at	3,08	up	CN181757	NP_566562.1	3E-61	dihydrolipoamide dehydrogenase 1, plastidic / lipoamide dehydrogenase 1 (PTLPD1)
Cit.16644.1.S1_at	3,34	up	CX635236	AAM28624.1	2E-99	adenosine monophosphate binding protein 7 AMPBP7 [Arabidopsis thaliana]
Cit.12140.1.S1_at	4,09	up	CF831272	NP_187498.1	1E-101	alanine--glyoxylate aminotransferase, putative / beta-alanine-pyruvate aminotransferase,
Cit.22652.1.S1_s_at	3,22	up	CF837212	NP_188589.1	5E-44	amino acid permease family protein [Arabidopsis thaliana]
Cit.9229.1.S1_x_at	3,33	up	CV705279	CAA65585.1	1E-54	arginine decarboxylase [Vitis vinifera]
Cit.9230.1.S1_s_at	3,68	up	CV710869	CAA65585.1	1E-35	arginine decarboxylase [Vitis vinifera]
Cit.12448.1.S1_s_at	3,26	up	CX664230	CAB41123.1	1E-112	argininosuccinate synthase-like protein [Arabidopsis thaliana]
Cit.12448.1.S1_at	4,15	up	CX664230	CAB41123.1	1E-112	argininosuccinate synthase-like protein [Arabidopsis thaliana]
Cit.10060.1.S1_at	5,19	up	CX545818	O24661	1E-165	Asparagine synthetase [glutamine-hydrolyzing] (Glutamine-dependent asparagine synthetase)
Cit.10060.1.S1_s_at	3,17	up	CX545818	O24661	1E-165	Asparagine synthetase [glutamine-hydrolyzing] (Glutamine-dependent asparagine synthetase)
Cit.11175.1.S1_at	3,55	up	CK935221	NP_196578.1	3E-79	beta-hydroxyacyl-ACP dehydratase, putative [Arabidopsis thaliana]
Cit.23739.1.S1_at	4,29	up	CV705814	BAC53936.1	2E-45	chromomethylase-like protein [Nicotiana tabacum]
Cit.17004.1.S1_x_at	3,46	up	CK936487	AAM29150.1	0.0	citrus sucrose transporter 1 [Citrus sinensis]
Cit.4075.1.S1_s_at	3,25	up	CF417340	BAB10038.1	1E-114	dihydropyrimidinase [Arabidopsis thaliana]
Cit.11518.1.S1_at	4,24	up	CX049088	CAA74176.1	1E-95	enoyl-ACP reductase [Nicotiana tabacum]
Cit.2177.1.S1_s_at	3,66	up	DN621492	AAB02006.1	4E-64	epoxide hydrolase [Nicotiana tabacum]
Cit.14052.1.S1_s_at	5,37	up	CX674135	AAK94781.1	2E-96	gamma hydroxybutyrate dehydrogenase [Arabidopsis thaliana]
Cit.9625.1.S1_s_at	3,72	up	CX051466	XP_480437.1	4E-76	glucose-6-phosphate/phosphate translocator [Oryza sativa (japonica cultivar-group)]
Cit.9618.1.S1_s_at	3,67	up	CB290456	P32289	1E-116	Glutamine synthetase nodule isozyme (Glutamate--ammonia ligase)
Cit.28391.1.S1_s_at	3,42	up	CV706058	AAD49734.1	4E-21	glutamine synthetase precursor [Juglans nigra]
Cit.4033.1.S1_at	3,88	up	CX044030	NP_174469.1	1E-70	histidine biosynthesis bifunctional protein (HISIE) [Arabidopsis thaliana]
Cit.22297.1.S1_s_at	4,62	up	CX670490	AAR18374.1	3E-91	nucleobase-ascorbate transporter 12 [Arabidopsis thaliana]
Cit.15004.1.S1_at	3,27	up	CF834783	NP_568283.2	1E-101	oxoglutarate/malate translocator, putative [Arabidopsis thaliana]
Cit.28009.1.S1_at	9,18	up	CX671808	NP_173510.1	1E-55	phosphate transporter family protein [Arabidopsis thaliana]
Cit.32610.1.S1_at	6,36	up	CX292670	NP_683294.2	6E-64	phosphoglycerate/bisphosphoglycerate mutase family protein [Arabidopsis thaliana]
Cit.14013.1.S1_at	3,22	up	CX644086	AAL07040.1	7E-08	putative oligosaccharyl transferase STT3 [Arabidopsis thaliana]
Cit.12149.1.S1_s_at	3,51	up	CX676139	XP_467829.1	9E-74	putative phosphoglycerate mutase [Oryza sativa (japonica cultivar-group)]
Cit.16081.1.S1_at	3,74	up	CX071680	AAK44013.1	3E-64	putative serine carboxypeptidase II [Arabidopsis thaliana]
Cit.6930.1.S1_at	3,29	up	CX638019	CAA71816.1	2E-30	ribonucleotide reductase [Nicotiana tabacum]
Cit.5337.1.S1_s_at	4,82	up	CX074648	CAA71816.1	1E-133	ribonucleotide reductase [Nicotiana tabacum]
Cit.3663.1.S1_at	3,46	up	CN190545	BAB01287.1	4E-90	sucrose cleavage protein-like [Arabidopsis thaliana]
Cit.5168.1.S1_at	4,26	up	CX636014	NP_201319.1	1E-86	trehalose-6-phosphate phosphatase, putative [Arabidopsis thaliana]
Cit.23076.1.S1_at	4,08	up	CV716736	NP_484455.1	2E-43	tryptophan synthase alpha chain [Nostoc sp. PCC 7120]
Cit.30025.1.S1_at	3,48	up	CK937612	NP_196963.2	2E-39	isocitrate dehydrogenase, putative / NADP+ isocitrate dehydrogenase, putative

Cell division and morphogenesis

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.1187.1.S1_at	3,01	up	CX072370	NP_198122.1	8E-35	40S ribosomal protein S21 (RPS21C) [Arabidopsis thaliana]
Cit.37509.1.S1_at	3,03	up	CF417107	NP_195360.1	4E-21	ribosomal protein L12 family protein [Arabidopsis thaliana]
Cit.3688.1.S1_at	3,22	up	CX636885	AAF26138.1	4E-60	putative 60S ribosomal protein L18 [Arabidopsis thaliana]
Cit.1873.1.S1_at	3,24	up	CN183434	AAK25760.1	4E-58	ribosomal protein L33 [Castanea sativa]
Cit.4062.1.S1_at	3,30	up	CK938771	AAW50980.1	8E-49	ribosomal protein L36 [Triticum aestivum]
Cit.11893.1.S1_at	3,51	up	CF835099	CAA46927.1	1E-123	ribosomal protein S1 [Spinacia oleracea]
Cit.5035.1.S1_s_at	3,56	up	CX544386	AAT47818.1	1E-108	At1g64880 [Arabidopsis thaliana]
Cit.14842.1.S1_s_at	3,68	up	CV886715	AAM66949.1	1E-50	ribosomal protein S25 [Arabidopsis thaliana]
Cit.9917.1.S1_x_at	3,75	up	CK932685	O22518	3E-89	40S ribosomal protein SA (p40)

Cit.23161.1.S1_x_at	3,77	up	CX073553	NP_564605.2	4E-62	ribosomal protein L22 family protein [Arabidopsis thaliana]
Cit.9456.1.S1_at	3,80	up	CX052528	XP_475816.1	8E-58	putative 60S ribosomal L28 protein [Oryza sativa (japonica cultivar-group)]
Cit.1536.1.S1_x_at	3,83	up	CX044981	S28420	6E-68	ubiquitin / ribosomal protein CEP52 - wood tobacco
Cit.11893.1.S1_s_at	3,92	up	CF835099	CAA46927.1	1E-123	ribosomal protein S1 [Spinacia oleracea]
Cit.17672.1.S1_at	4,01	up	CB611006	AAF34800.1	1E-56	60S ribosomal protein L35 [Euphorbia esula]
Cit.14920.1.S1_at	4,04	up	CX051565	BAA96072.1	2E-24	ribosomal protein L29 [Panax ginseng]
Cit.23161.1.S1_at	4,17	up	CX073553	NP_564605.2	4E-62	ribosomal protein L22 family protein [Arabidopsis thaliana]
Cit.31075.1.S1_at	4,28	up	CF931497	NP_196435.1	3E-60	ribosomal protein L7Ae/L30e/S12e/Gadd45 family protein [Arabidopsis thaliana]
Cit.31075.1.S1_s_at	4,50	up	CF931497	NP_196435.1	3E-60	ribosomal protein L7Ae/L30e/S12e/Gadd45 family protein [Arabidopsis thaliana]
Cit.23200.1.S1_at	4,77	up	CX076016	CAB93719.1	2E-58	nhp2-like protein [Arabidopsis thaliana] ref|NP_196435.1|
Cit.8891.1.S1_at	5,15	up	CV708274	NP_909841.1	1E-111	ribosomal protein L15 [Oryza sativa (japonica cultivar-group)]
Cit.1359.1.S1_at	3,40	up	CX297571	AAP68880.1	3E-27	putative ribosomal protein S29 [Oryza sativa (japonica cultivar-group)]
Cit.8868.1.S1_at	3,01	up	CN189718	BAB86847.1	1E-157	elongation factor EF-2 [Pisum sativum]
Cit.24015.1.S1_x_at	3,11	up	CV711510	AAL79775.1	8E-39	elongation factor 1 alpha [Saccharum hybrid cultivar CP72-2086]
Cit.2435.1.S1_s_at	6,06	up	CX667512	CAA61511.1	1E-150	mitochondrial elongation factor Tu [Arabidopsis thaliana]
Cit.4723.1.S1_at	3,11	up	CV884884	AAM64788.1	4E-65	histone H2A.F/Z [Arabidopsis thaliana]
Cit.17322.1.S1_s_at	4,95	up	CK935733	CAA07234.1	9E-58	histone H2A [Cicer arietinum]
Cit.1937.1.S1_s_at	5,14	up	CX643907	NP_910496.1	4E-36	histone H3 [Oryza sativa (japonica cultivar-group)]
Cit.1938.1.S1_at	7,21	up	CK932856	CAD41584.3	2E-69	histone H3 [Oryza sativa (japonica cultivar-group)]
Cit.11496.1.S1_s_at	7,32	up	CK933242	AAF65769.1	3E-66	histone H2A [Euphorbia esula]
Cit.4118.1.S1_s_at	7,52	up	CK932935	CAE02924.1	2E-69	histone H3 [Oryza sativa (japonica cultivar-group)]
Cit.11497.1.S1_at	9,71	up	CX667228	AAF65769.1	9E-67	histone H2A [Euphorbia esula]
Cit.5320.1.S1_s_at	3,12	up	CX666028	BAD68466.1	4E-93	putative kinesin [Oryza sativa (japonica cultivar-group)]
Cit.6842.1.S1_at	3,13	up	CV712960	BAB10642.1	2E-47	kinesin-like protein [Arabidopsis thaliana]
Cit.16404.1.S1_at	3,17	up	CD575625	NP_190059.3	9E-36	kinesin motor protein-related [Arabidopsis thaliana]
Cit.26988.1.S1_at	3,21	up	CV716466	AAN13032.1	5E-41	putative kinesin protein [Arabidopsis thaliana]
Cit.15707.1.S1_at	3,28	up	CF828325	BAB40710.1	5E-20	BY-2 kinesin-like protein 10 [Nicotiana tabacum]
Cit.22706.1.S1_at	3,31	up	CN184192	F84614	1E-14	probable kinesin heavy chain [imported] [Arabidopsis thaliana]
Cit.23669.1.S1_at	3,44	up	CV705097	AAC98061.1	7E-32	putative kinesin heavy chain [Arabidopsis thaliana]
Cit.29883.1.S1_a_at	3,75	up	CF510057	NP_190059.3	2E-63	kinesin motor protein-related [Arabidopsis thaliana]
Cit.12868.1.S1_s_at	3,87	up	CX667309	O23826	3E-98	125 kDa kinesin-related protein
Cit.12868.1.S1_at	4,17	up	CX667309	O23826	3E-98	125 kDa kinesin-related protein pir||T02017 kinesin-related protein
Cit.32235.1.S1_at	4,24	up	CX289519	AAO15358.1	9E-45	kinesin related protein [Lycopersicon esculentum]
Cit.21989.1.S1_at	4,24	up	DN622724	BAB40709.1	4E-21	BY-2 kinesin-like protein 5 [Nicotiana tabacum]
Cit.13867.1.S1_at	4,28	up	CX053924	BAA01972.1	1E-105	kinesin-like motor protein heavy chain [Arabidopsis thaliana]
Cit.15144.1.S1_s_at	4,57	up	CX666706	AAO42115.1	1E-51	putative kinesin [Arabidopsis thaliana]
Cit.28882.1.S1_s_at	3,22	up	CX544918	AAM65411.1	1E-17	tubulin beta-2/beta-3 chain [Arabidopsis thaliana]
Cit.1919.1.S1_s_at	3,49	up	CX672740	AAL92118.1	1E-151	beta-tubulin [Gossypium hirsutum]
Cit.9005.1.S1_s_at	4,30	up	CV708972	NP_912596.1	1E-166	tubulin beta-4 chain [Oryza sativa (japonica cultivar-group)]
Cit.2014.1.S1_s_at	4,90	up	CX076714	AAB03267.1	1E-171	beta-tubulin 2 sp|Q40106|TBB2_LUPAL Tubulin beta-2 chain (Beta-2 tubulin)
Cit.12899.1.S1_s_at	5,31	up	CV719766	AAQ92663.1	1E-145	alpha-tubulin 4 [Gossypium hirsutum]
Cit.28866.1.S1_s_at	5,39	up	CX544136	CAA38615.1	2E-32	beta-tubulin 3 [Pisum sativum]
Cit.18069.1.S1_at	3,68	up	DN799208	AAG50105.1	5E-51	putative tubulin alpha-6 chain [Arabidopsis thaliana]
Cit.8253.1.S1_s_at	3,68	up	CK935251	T51176	1E-151	actin [imported] - mung bean gb|AAF31643.1| actin [Vigna radiata]
Cit.14570.1.S1_s_at	4,25	up	CX077118	AAM65311.1	2E-70	microtubule-associated protein EB1-like protein [Arabidopsis thaliana]
Cit.31179.1.S1_at	3,82	up	CX304928	XP_475231.1	2E-75	putative microtubule-associated protein [Oryza sativa (japonica cultivar-group)]
Cit.30293.1.S1_at	3,91	up	DN798669	AAT40494.1	1E-79	putative microtubule-associated protein [Solanum demissum]
Cit.3952.1.S1_at	3,23	up	CX301585	AAM66101.1	1E-106	chaperonin subunit, putative [Arabidopsis thaliana]
Cit.4597.1.S1_at	3,12	up	DN622980	NP_567757.1	1E-71	co-chaperone grpE family protein [Arabidopsis thaliana]
Cit.40357.1.S1_s_at	3,25	up	DR403353	AAM64345.1	5E-29	heat shock protein-like [Arabidopsis thaliana]
Cit.15338.1.S1_at	3,29	up	CN188518	CAA12387.1	2E-42	Hsp20.1 protein [Lycopersicon peruvianum]
Cit.15412.1.S1_at	3,30	up	CX306562	CAA82945.1	5E-81	heat-shock protein [Secale cereale]
Cit.35378.1.S1_s_at	4,31	up	CK938091	NP_179646.1	2E-96	DNAJ heat shock family protein [Arabidopsis thaliana]
Cit.31059.1.S1_at	4,42	up	DN795229	NP_178487.1	6E-92	heat shock protein, putative [Arabidopsis thaliana]
Cit.33267.1.S1_x_at	4,99	up	CX299131	AAM64345.1	4E-26	heat shock protein-like [Arabidopsis thaliana]
Cit.8021.1.S1_x_at	3,41	up	CK933174	AAN86274.1	6E-62	non-cell-autonomous heat shock cognate protein 70 [Cucurbita maxima]
Cit.26707.1.S1_s_at	3,30	up	CX046580	AAR07942.1	3E-71	DNA gyrase A subunit [Nicotiana benthamiana]
Cit.31249.1.S1_at	3,40	up	CX048395	AAC97224.1	2E-37	putative helicase [Arabidopsis thaliana]
Cit.13586.1.S1_at	3,32	up	CF834266	NP_190563.1	4E-49	LOB domain protein 38 / lateral organ boundaries domain protein 38 (LBD38) [Arabidopsis thaliana]
Cit.7588.1.S1_at	3,33	up	CV716571	BAC15746.1	2E-50	B1 type cyclin [Daucus carota]
Cit.26326.1.S1_s_at	3,48	up	CV885476	NP_922089.1	6E-97	putative DNA ligase [Oryza sativa (japonica cultivar-group)]
Cit.6674.1.S1_at	3,56	up	CV884711	BAA20412.1	3E-88	A-type cyclin [Catharanthus roseus]

Cit.15333.1.S1_at	3,56	up	CX051359	NP_973836.1	4E-53	transducin family protein / WD-40 repeat family protein [Arabidopsis thaliana]
Cit.6613.1.S1_at	3,61	up	CN189671	NP_851242.1	8E-91	brix domain-containing protein [Arabidopsis thaliana]
Cit.23460.1.S1_at	3,62	up	CX674332	BAC42527.1	1E-107	putative WD-repeat protein [Arabidopsis thaliana]
Cit.859.1.S1_s_at	3,67	up	CX643445	NP_568772.3	1E-103	fibrillarin 1 (FBR1) (FIB1) (SKIP7) [Arabidopsis thaliana]
Cit.28338.1.S1_at	4,52	up	DN621460	AAN13105.1	9E-72	fibrillarin 2 (AtFib2) [Arabidopsis thaliana]
Cit.13967.1.S1_s_at	3,76	up	CN181570	T03582	3E-91	probable replication protein A1 - rice gb|AAB71836.1| replication protein A1 [Oryza sativa]
Cit.13968.1.S1_at	3,67	up	CN190507	AAB71836.1	9E-57	replication protein A1 [Oryza sativa]
Cit.39560.1.S1_at	3,71	up	DN795571	NP_178186.1	1E-69	transducin family protein / WD-40 repeat family protein [Arabidopsis thaliana]
Cit.23379.1.S1_s_at	3,77	up	CX672446	CAB79839.1	2E-51	DNA topoisomerase like-protein [Arabidopsis thaliana]
Cit.7073.1.S1_at	3,90	up	DN618785	T07737	1E-108	dem protein - tomato emb|CAA73973.1| dem [Lycopersicon esculentum]
Cit.4666.1.S1_at	4,05	up	CV706955	AAO23606.1	1E-102	At2g27920/T1E2.16 [Arabidopsis thaliana]
Cit.38876.1.S1_at	4,13	up	CK740159	NP_190761.1	3E-23	transducin family protein / WD-40 repeat family protein [Arabidopsis thaliana]
Cit.7830.1.S1_at	4,26	up	CV718826	AAQ89630.1	1E-102	cell cycle checkpoint protein MAD2-like [Arabidopsis thaliana]
Cit.5864.1.S1_at	4,30	up	CX070958	CAA44188.1	3E-93	mitotic cyclin [Glycine max]
Cit.4250.1.S1_s_at	4,38	up	CV713016	AAK07610.1	1E-125	prohibitin 1-like protein [Brassica napus]
Cit.12502.1.S1_s_at	4,39	up	CX050861	AAT39315.1	2E-90	putative senescence-associated protein [Solanum demissum]
Cit.6836.1.S1_at	4,47	up	CV717182	AAS68103.1	1E-119	minichromosomal maintenance factor [Triticum aestivum]
Cit.7153.1.S1_at	4,92	up	CV884591	CAA10166.1	5E-64	MCM3 protein [Pisum sativum]
Cit.27164.1.S1_s_at	4,51	up	CV718768	T07675	1E-90	cyclin a2-type, mitosis-specific - soybean dbj|BAA09466.1| mitotic cyclin a2-type [Glycine max]
Cit.12503.1.S1_at	4,84	up	DN135183	NP_190146.1	3E-82	senescence-associated family protein [Arabidopsis thaliana]
Cit.6867.1.S1_at	5,09	up	CX675841	AAQ08180.1	1E-20	TK1-like deoxyribonucleoside kinase [Lycopersicon esculentum]
Cit.4304.1.S1_s_at	5,35	up	CX077265	BAD03017.1	2E-38	putative RNA recognition motif (RRM)-containing protein [Oryza sativa (japonica cultivar-group)]
Cit.3941.1.S1_at	5,48	up	CN190662	AAC49690.1	1E-114	prohibitin [Nicotiana tabacum]
Cit.6285.1.S1_at	5,51	up	CX673906	AAP58374.1	7E-73	RPA 32kDa [Pisum sativum]
Cit.13956.1.S1_at	5,67	up	CN182148	AAP73784.1	1E-126	cyclin-dependent kinase [Populus tremula x Populus tremuloides]
Cit.9905.1.S1_s_at	5,77	up	CX672038	AAQ23176.1	1E-105	subtilisin-like protease [Glycine max]
Cit.14181.1.S1_at	5,89	up	CF417611	T14286	4E-82	embryogenic callus protein 98b - carrot dbj|BAA32827.1| 98b [Daucus carota]
Cit.20602.1.S1_at	6,55	up	CK740082	NP_911358.1	6E-08	putative snRNP protein [Oryza sativa (japonica cultivar-group)]
Cit.17811.1.S1_at	6,60	up	CF508134	AAP83582.1	1E-42	phosphoethanolamine N-methyltransferase [Brassica napus]
Cit.14761.1.S1_at	7,00	up	CX071202	AAC26671.1	1E-118	putative DNA replication licensing factor, mcm5 [Arabidopsis thaliana]
Cit.33111.1.S1_at	7,17	up	CX297398	AAQ72567.1	2E-38	mini-chromosome maintenance 7 [Pisum sativum]
Cit.10585.1.S1_s_at	8,80	up	CX673652	AAM91745.1	6E-93	putative phosphoethanolamine N-methyltransferase [Arabidopsis thaliana]
Cit.18235.1.S1_at	9,48	up	CK936113	NP_187298.1	2E-92	GNS1/SUR4 membrane family protein [Arabidopsis thaliana]
Cit.5956.1.S1_at	10,38	up	CV885460	AAM18791.1	8E-31	immuno-reactant natriuretic peptide-like protein [Erucastrum strigosum]
Cit.5300.1.S1_at	11,71	up	CV713508	AAL34163.1	5E-70	putative dUTP pyrophosphatase [Arabidopsis thaliana]
Cit.3665.1.S1_at	11,84	up	CF508354	CAB45241.1	7E-34	GEG protein [Gerbera hybrid cultivar]
Cit.7008.1.S1_at	3,10	up	CX288730	AAQ14331.1	1E-65	MYC1 [Catharanthus roseus]
Cit.5975.1.S1_at	3,17	up	CX049547	XP_479287.1	2E-70	putative protein arginine N-methyltransferase 3 [Oryza sativa (japonica cultivar-group)]
Cit.27008.1.S1_s_at	3,17	up	CV716762	NP_190146.1	1E-103	senescence-associated protein 5-like protein [Arabidopsis thaliana]
Cit.17986.1.S1_s_at	3,28	up	CX640376	AAM16254.1	2E-33	AT3g45010/F14D17_80 [Arabidopsis thaliana]

Cell-wall synthesis and remodelling

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.11221.1.S1_at	3,03	up	CV709262	AAD30579.1	1E-87	Similar to dTDP-D-glucose 4,6-dehydratase [Arabidopsis thaliana]
Cit.29819.1.S1_at	3,04	up	CF418485	BAB01763.1	2E-85	beta-1,3-glucanase-like protein [Arabidopsis thaliana]
Cit.13509.1.S1_at	3,05	up	CX671694	NP_201416.1	6E-97	glycosyl hydrolase family 38 protein [Arabidopsis thaliana]
Cit.15463.1.S1_at	3,09	up	CX050368	CAA42942.1	1E-05	proline-rich protein [Phaseolus vulgaris]
Cit.17023.1.S1_s_at	3,14	up	CK936315	NP_201042.2	3E-54	invertase/pectin methylesterase inhibitor family protein [Arabidopsis thaliana]
Cit.14005.1.S1_s_at	3,14	up	CV710432	AAK48848.1	1E-101	expansin [Prunus cerasus]
Cit.13098.1.S1_at	3,15	up	CD575378	NP_188636.1	1E-145	aspartyl protease family protein [Arabidopsis thaliana]
Cit.1562.1.S1_at	3,15	up	CX673052	AAM61180.1	7E-99	contains similarity to endo-1,3-1,4-beta-D-glucanase [Arabidopsis thaliana]
Cit.29616.1.S1_s_at	3,27	up	CV884354	CAA03846.1	2E-28	starch branching enzyme II, SBE-II [Solanum tuberosum]
Cit.3601.1.S1_at	3,29	up	CV705154	AAF70825.1	1E-129	putative beta-galactosidase [Lycopersicon esculentum]
Cit.23967.1.S1_s_at	3,31	up	CV710106	CAA10382.2	4E-60	alpha-D-xylosidase [Tropaeolum majus]
Cit.13252.1.S1_at	3,40	up	CD573747	AAD12577.1	1E-130	putative cellulase [Fragaria x ananassa]
Cit.22527.1.S1_s_at	3,40	up	CF832471	CAA65634.1	1E-28	PS60 [Nicotiana tabacum]
Cit.6652.1.S1_at	3,41	up	CX665366	AAT64028.1	1E-119	cellulose synthase [Gossypium hirsutum]
Cit.2032.1.S1_s_at	3,47	up	CX667126	BAA78708.1	1E-70	beta-glucosidase [Polygonum tinctorium]
Cit.7299.1.S1_s_at	3,54	up	CX543131	T09854	4E-26	proline-rich cell wall protein - upland cotton
Cit.38806.1.S1_at	3,56	up	CK702487	AAL38529.1	6E-57	CSLD4 [Oryza sativa]
Cit.24023.1.S1_s_at	3,57	up	CV711956	AAQ87025.1	6E-89	pectate lyase-like protein [Brassica napus]
Cit.17757.1.S1_at	3,58	up	BQ623570	AAF25357.1	2E-76	dirigent protein [Forsythia x intermedia]
Cit.374.1.S1_s_at	3,60	up	DN959507	AAR09170.1	1E-126	alpha-expansin 3 [Populus tremula x Populus tremuloides]

Cit.14522.1.S1_at	3,60	up	CX043829	CAA10382.2	1E-82	alpha-D-xylosidase [Tropaeolum majus]
Cit.311.1.S1_s_at	3,64	up	CN182741	BAB16431.1	8E-18	P-rich protein NtEIG-C29 [Nicotiana tabacum]
Cit.29537.1.S1_at	3,70	up	CV709330	AAM75139.1	1E-126	alkaline alpha galactosidase I [Cucumis melo]
Cit.29327.1.S1_at	3,71	up	CK934531	AAR09170.1	1E-72	alpha-expansin 3 [Populus tremula x Populus tremuloides]
Cit.30289.1.S1_at	3,72	up	CX288295	AAL90992.1	5E-82	brassinosteroid signalling positive regulator-related [Arabidopsis thaliana]
Cit.30858.1.S1_at	3,75	up	CF505371	BAC67194.1	2E-40	expansin [Pyrus communis]
Cit.399.1.S1_s_at	3,81	up	CF837795	AAR09170.1	2E-45	alpha-expansin 3 [Populus tremula x Populus tremuloides]
Cit.14122.1.S1_at	3,82	up	CK665535	BAC78828.1	2E-93	caffeic acid O-methyltransferase [Rosa chinensis var. spontanea]
Cit.29026.1.S1_at	3,97	up	CX638097	CAA50561.1	3E-45	catechol O-methyltransferase [Nicotiana tabacum]
Cit.18784.1.S1_at	4,01	up	CX301016	CAE51357.1	7E-27	putative polygalacturonase [Musa acuminata]
Cit.12573.1.S1_at	4,08	up	CX673518	NP_189269.2	7E-46	glycosyl hydrolase family protein 27 / alpha-galactosidase family protein / melibiase family protein
Cit.19350.1.S1_s_at	4,12	up	CK934803	NP_196320.1	4E-42	aspartyl protease family protein [Arabidopsis thaliana]
Cit.14266.1.S1_at	4,17	up	CX666282	CAB88664.1	1E-164	putative glucosyltransferase [Cicer arietinum]
Cit.14005.1.S1_at	4,17	up	CV710432	AAK48848.1	1E-101	expansin [Prunus cerasus]
Cit.4284.1.S1_at	4,22	up	CV714792	AAM65039.1	4E-95	putative glucan endo-1-3-beta-glucosidase [Arabidopsis thaliana]
Cit.3070.1.S1_s_at	4,28	up	CF830964	AAN32954.1	2E-67	alkaline alpha-galactosidase seed imbibition protein [Lycopersicon esculentum]
Cit.2001.1.S1_at	4,29	up	CV884879	BAA81686.1	9E-59	Arabinogalactan protein [Cucumis sativus]
Cit.9664.1.S1_s_at	4,30	up	CX663555	S65063	2E-39	fiber protein E6 (clones SIE6-2A and SIE6-3B) -
Cit.1077.1.S1_s_at	4,31	up	CF829440	AAQ84042.1	1E-93	pectate lyase [Malus x domestica]
Cit.5156.1.S1_at	4,33	up	CX048605	CAB79832.1	1E-104	1, 3-beta-glucanase-like protein [Arabidopsis thaliana]
Cit.27919.1.S1_s_at	4,34	up	CX049839	NP_851092.1	2E-87	glycosyl hydrolase family 79 N-terminal domain-containing protein [Arabidopsis thaliana]
Cit.2949.1.S1_s_at	4,49	up	CN182557	T10523	1E-153	xyloglucan endo-1,4-beta-D-glucanase (EC 3.2.1.-) 1
Cit.24163.1.S1_s_at	4,55	up	CD575247	BAA89481.1	3E-71	beta-1,3-glucanase [Salix gilgiana]
Cit.20524.1.S1_at	4,71	up	CF653393	NP_177041.2	4E-04	arabinogalactan-protein, putative (AGP19) [Arabidopsis thaliana]
Cit.30534.1.S1_s_at	5,18	up	CK934786	S65063	4E-21	fiber protein E6 (clones SIE6-2A and SIE6-3B) -
Cit.7877.1.S1_at	5,19	up	CX667721	S48032	5E-95	cim1 protein - soybean gb|AAA50175.1| cytokinin induced message
Cit.13732.1.S1_at	5,42	up	CB293314	AAB63624.1	5E-47	cellulose synthase isolog [Arabidopsis thaliana]
Cit.1385.1.S1_s_at	5,42	up	CV887009	T10737	7E-67	extensin-like cell wall protein - sea-island cotton
Cit.21778.1.S1_at	5,48	up	CX306527	AAP04151.1	4E-09	putative glycosyl hydrolase family 17 protein (beta-1,3-glucanase bg3) [Arabidopsis thaliana]
Cit.17853.1.S1_s_at	5,50	up	CK933446	NP_563715.1	1E-145	pectate lyase family protein [Arabidopsis thaliana]
Cit.15597.1.S1_at	5,58	up	CN183165	NP_568431.1	6E-61	high mobility group (HMG1/2) family protein [Arabidopsis thaliana]
Cit.21769.1.S1_at	5,64	up	CX306275	AAP37856.1	1E-54	alpha-galactosidase-like protein [Arabidopsis thaliana]
Cit.3876.1.S1_s_at	5,66	up	CX051837	AAT37954.1	3E-73	fasciclin-like AGP 11 [Populus alba x Populus tremula]
Cit.2945.1.S1_s_at	5,74	up	CV887291	CAC94006.1	1E-148	endo-beta-1,4-glucanase [Fragaria x ananassa]
Cit.26012.1.S1_at	5,84	up	CF833607	AAF35897.1	8E-56	pectin methylesterase isoform alpha [Vigna radiata]
Cit.28883.1.S1_at	5,87	up	CX544928	AAS55083.1	9E-54	UDP-glucose glucosyltransferase [Rhodiola sachalinensis]
Cit.1558.1.S1_at	6,01	up	CX663363	BAB02778.1	1E-67	contains similarity to endo-1,3-1,4-beta-D-glucanase [Arabidopsis thaliana]
Cit.9704.1.S1_at	6,13	up	CF836817	CAA03908.1	1E-108	beta-1,3-glucanase [Citrus sinensis]
Cit.8725.1.S1_at	6,26	up	CX043719	AAL91506.1	1E-94	caffeic acid O-methyltransferase II [Nicotiana tabacum]
Cit.20223.1.S1_x_at	6,59	up	CX045519	AAL91506.1	9E-73	caffeic acid O-methyltransferase II [Nicotiana tabacum]
Cit.2701.1.S1_at	6,67	up	CV710968	AAO92753.1	2E-56	arabinogalactan protein [Gossypium hirsutum]
Cit.12820.1.S1_at	6,68	up	CK665203	AAU05497.1	3E-89	pectinacetylesterase, putative [Arabidopsis thaliana]
Cit.1386.1.S1_at	6,89	up	CX672178	T10737	5E-75	extensin-like cell wall protein - sea-island cotton gb|AAA79364.1| proline-rich cell wall protein
Cit.3554.1.S1_s_at	6,94	up	CX663293	T07885	1E-124	cellulase (EC 3.2.1.4) - sweet orange gb|AAB65156.1| basic cellulase [Citrus sinensis]
Cit.30472.1.S1_at	6,96	up	AJ489039	Q9FQY8	3E-11	Caffeic acid 3-O-methyltransferase (S-adenosysl-L-methionine:caffeic acid 3-O-methyltransferase)
Cit.4516.1.S1_s_at	7,36	up	CV886618	AAP33475.1	1E-116	polygalacturonase-like protein [Fragaria x ananassa]
Cit.11915.1.S1_s_at	7,47	up	CX071344	AAC17605.1	6E-45	Contains similarity to proline-rich protein,
Cit.1322.1.S1_s_at	7,49	up	CN192282	NP_173653.1	3E-87	UDP-glucoronosyl/UDP-glucosyl transferase family protein [A. thaliana]
Cit.20158.1.S1_x_at	7,61	up	CF834901	NP_191008.1	7E-23	aspartyl protease family protein [Arabidopsis thaliana]
Cit.2700.1.S1_s_at	7,80	up	CV709336	AAO92753.1	7E-81	arabinogalactan protein [Gossypium hirsutum]
Cit.20223.1.S1_at	8,36	up	CX045519	AAL91506.1	9E-73	caffeic acid O-methyltransferase II [Nicotiana tabacum]
Cit.2738.1.S1_s_at	8,40	up	CN182109	CAD22154.1	4E-80	pherophorin-dz1 protein [Volvox carteri f. nagariensis]
Cit.11898.1.S1_at	8,43	up	CD573639	AAL57631.1	4E-69	glycosyl hydrolase family 3 protein [Arabidopsis thaliana]
Cit.17888.1.S1_s_at	8,51	up	CF838857	NP_177929.1	1E-106	glycosyl hydrolase family 3 protein [Arabidopsis thaliana]
Cit.13410.1.S1_s_at	9,14	up	CX669534	AAP04044.1	1E-103	putative pectin methylesterase [Arabidopsis thaliana]
Cit.1561.1.S1_s_at	9,81	up	CV714253	AAM61180.1	5E-94	contains similarity to endo-1,3-1,4-beta-D-glucanase [Arabidopsis thaliana]
Cit.35754.1.S1_at	10,44	up	CB250305	NP_191544.1	2E-09	polygalacturonase, putative [Arabidopsis thaliana]
Cit.35756.1.S1_at	10,54	up	CB250319	AAC64184.1	2E-96	endopolygalacturonase [Prunus persica]
Cit.11685.1.S1_s_at	11,28	up	CX663825	AAM65121.1	5E-74	putative proline-rich cell wall protein [Arabidopsis thaliana]
Cit.32767.1.S1_at	11,70	up	CX294670	AAP21999.1	1E-58	endopolygalacturonase [Prunus persica]
Cit.3390.1.S1_at	12,52	up	CV709535	NP_197666.1	1E-128	glycosyl transferase family 2 protein [Arabidopsis thaliana]
Cit.4483.1.S1_s_at	14,46	up	CX077158	BAB11424.1	5E-79	beta-xylosidase [Arabidopsis thaliana] ref|NP_201262.1|

Cit.17291.1.S1_at	15,92	up	CX302017	AAF60951.1	3E-77	O-methyltransferase [Populus balsamifera subsp. trichocarpa x Populus deltoides]
Cit.25554.1.S1_at	16,24	up	CX669376	AAK50769.1	1E-63	polygalacturonase [Pisum sativum]
Cit.8718.1.S1_s_at	19,42	up	CX670983	CAA50561.1	2E-99	catechol O-methyltransferase [Nicotiana tabacum]
Cit.20071.1.S1_s_at	20,93	up	CX666732	AAP33475.1	4E-90	polygalacturonase-like protein [Fragaria x ananassa]
Cit.12490.1.S1_s_at	22,09	up	CX044619	AAM20001.1	1E-134	putative polygalacturonase PG1 [Arabidopsis thaliana]
Cit.9706.1.S1_s_at	23,17	up	CV886686	CAA03908.1	1E-145	beta-1,3-glucanase [Citrus sinensis]
Cit.10437.1.S1_s_at	33,82	up	CX665316	AAP33475.1	1E-144	polygalacturonase-like protein [Fragaria x ananassa]
Cit.21559.1.S1_s_at	34,76	up	CX051882	AAP33475.1	4E-33	polygalacturonase-like protein [Fragaria x ananassa]
Cit.2392.1.S1_at	42,38	up	CF831790	AAB65155.1	0.0	acidic cellulase [Citrus sinensis]

Disease resistance, defence and stress response

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.5743.1.S1_s_at	3,01	up	CX643231	AAD11484.1	3E-44	peroxidase [Glycine max]
Cit.17781.1.S1_at	3,02	up	DN799310	CAA55862.1	1E-29	ubiquinol--cytochrome c reductase [Solanum tuberosum]
Cit.11854.1.S1_at	3,04	up	CX046093	CAA71492.1	5E-98	peroxidase [Spinacia oleracea]
Cit.25478.1.S1_at	3,05	up	CX666877	AAP03018.1	1E-100	4-coumarate-CoA ligase-like protein [Arabidopsis thaliana]
Cit.2538.1.S1_at	3,05	up	CF837686	NP_181526.1	2E-40	peroxisomal membrane protein (PMP36) [Arabidopsis thaliana]
Cit.17961.1.S1_s_at	3,05	up	DN798478	BAB16427.1	4E-12	NtEIG-E80 [Nicotiana tabacum]
Cit.20982.1.S1_s_at	3,06	up	DN795079	BAB09573.1	8E-04	AAA-type ATPase-like protein [Arabidopsis thaliana]
Cit.19053.1.S1_at	3,07	up	CX302166	AAV34889.1	9E-26	osmotin-like [Theobroma cacao]
Cit.26764.1.S1_at	3,08	up	CV713176	BAD29345.1	7E-19	zinc finger protein-like [Oryza sativa (japonica cultivar-group)]
Cit.2718.1.S1_s_at	3,08	up	CK939764	AAC61283.1	8E-81	22 kDa peroxisomal membrane protein [Arabidopsis thaliana]
Cit.37302.1.S1_at	3,10	up	BQ624679	NP_189443.2	5E-61	leucine-rich repeat transmembrane protein kinase, putative [Arabidopsis thaliana]
Cit.31341.1.S1_at	3,10	up	CX297758	BAC21263.1	2E-51	glutathione S-transferase [Cucurbita maxima]
Cit.10645.1.S1_at	3,10	up	CX664652	AAN28853.1	2E-32	At4g27500/F27G19_100 [Arabidopsis thaliana]
Cit.6048.1.S1_s_at	3,11	up	CV715327	AAF61863.1	3E-40	DNA-binding protein 3 [Nicotiana tabacum]
Cit.17765.1.S1_x_at	3,12	up	CX304476	CAA48027.1	7E-05	Eli3-1 [Arabidopsis thaliana]
Cit.6998.1.S1_at	3,12	up	CX043364	AAR13301.1	3E-35	anthocyanin acyltransferase [Phaseolus vulgaris]
Cit.9587.1.S1_at	3,13	up	CX293561	AAG30140.1	7E-82	glutathione S-transferase [Arabidopsis thaliana]
Cit.2538.1.S1_s_at	3,13	up	CF837686	NP_181526.1	2E-40	peroxisomal membrane protein (PMP36) [Arabidopsis thaliana]
Cit.19486.1.S1_at	3,13	up	CK933061	NP_849378.1	1E-44	peroxisomal membrane protein-related [Arabidopsis thaliana]
Cit.5309.1.S1_at	3,15	up	CV717349	NP_199935.2	7E-86	ubiquinol-cytochrome C chaperone family protein [Arabidopsis thaliana]
Cit.12475.1.S1_at	3,16	up	CX640275	CAA54613.1	3E-92	UTP-glucose glucosyltransferase [Manihot esculenta]
Cit.31138.1.S1_at	3,17	up	CN184779	NP_200184.1	2E-73	harpin-induced family protein / HIN1 family protein / harpin-responsive family protein [Arabidopsis thaliana]
Cit.17228.1.S1_at	3,19	up	CV715877	AAB36652.1	1E-101	immediate-early salicylate-induced glucosyltransferase
Cit.11856.1.S1_at	3,19	up	AY243478	AAO72741.1	0.0	allene oxide synthase [Citrus sinensis]
Cit.25974.1.S1_at	3,20	up	CF832469	NP_177466.1	7E-22	nascent polypeptide-associated complex (NAC) domain-containing protein [Arabidopsis thaliana]
Cit.17762.1.S1_at	3,22	up	CN183091	BAB84352.1	3E-44	lipoxygenase [Citrus jambhiri]
Cit.32625.1.S1_s_at	3,22	up	CX292843	CAA58733.1	4E-07	PAR-1a [Nicotiana tabacum]
Cit.27321.1.S1_at	3,22	up	CX070547	AAN28917.1	3E-61	At4g25340/T30C3_20 [Arabidopsis thaliana]
Cit.28036.1.S1_s_at	3,22	up	CX672359	BAB01326.1	4E-94	receptor-like kinase [Arabidopsis thaliana]
Cit.17265.1.S1_at	3,24	up	CK937355	BAA92155.1	2E-19	glycine-rich protein [Citrus unshiu]
Cit.10638.1.S1_at	3,26	up	CV884532	NP_564149.1	1E-106	20S proteasome beta subunit C1 (PBC1) (PRCT) [Arabidopsis thaliana]
Cit.37306.1.S1_at	3,27	up	BQ624718	NP_193956.2	1E-08	zinc finger (C3HC4-type RING finger) protein family [Arabidopsis thaliana]
Cit.4177.1.S1_at	3,30	up	CX047099	AAO50725.1	1E-102	putative lipase [Arabidopsis thaliana]
Cit.11356.1.S1_s_at	3,31	up	CF837067	AAM73656.1	4E-80	AER [Nicotiana tabacum]
Cit.13716.1.S1_s_at	3,31	up	DN625547	CAB42912.1	2E-85	putative cold acclimation protein [Arabidopsis thaliana]
Cit.304.1.S1_s_at	3,31	up	BQ623782	CAE53882.1	1E-142	aquaporin [Ricinus communis]
Cit.6937.1.S1_at	3,33	up	CX546610	AAL25542.1	4E-12	At1g73800/F25P22_22 [Arabidopsis thaliana]
Cit.16551.1.S1_at	3,34	up	CV705388	DAA00872.1	3E-57	TPA: PDR4 ABC transporter [Arabidopsis thaliana]
Cit.15757.1.S1_at	3,35	up	CX077288	NP_182100.2	5E-08	BON1-associated protein (BAP1)-related [Arabidopsis thaliana]
Cit.6660.1.S1_at	3,37	up	CX306249	CAC18730.1	1E-100	NADH glutamate dehydrogenase [Vitis vinifera]
Cit.5206.1.S1_at	3,38	up	CX545682	NP_200956.1	6E-72	leucine-rich repeat transmembrane protein kinase, putative [Arabidopsis thaliana]
Cit.10716.1.S1_at	3,38	up	CV713082	NP_568984.1	4E-43	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein [Arabidopsis thaliana]
Cit.1820.1.S1_at	3,40	up	DN797852	BAA21921.1	9E-37	ZPT2-12 [Petunia x hybrida]
Cit.26982.1.S1_at	3,42	up	CV716390	NP_189066.1	1E-92	leucine-rich repeat transmembrane protein kinase, putative [Arabidopsis thaliana]
Cit.425.1.S1_s_at	3,44	up	CX047220	CAA80963.1	4E-46	blue copper protein [Pisum sativum]
Cit.7331.1.S1_at	3,44	up	CK665301	AAS80149.1	2E-49	ACT11D09.3 [Cucumis melo]
Cit.3236.1.S1_at	3,46	up	CK939355	NP_568568.1	5E-39	calcium-binding EF hand family protein [Arabidopsis thaliana]
Cit.5664.1.S1_at	3,47	up	CX671291	AAT28308.1	1E-67	leucine-rich repeat receptor-like protein kinase [Pyrus pyrifolia]
Cit.12803.1.S1_s_at	3,49	up	DN625595	BAB02040.1	1E-102	serine/threonine kinase [Arabidopsis thaliana]
Cit.5206.1.S1_s_at	3,52	up	CX545682	NP_200956.1	6E-72	leucine-rich repeat transmembrane protein kinase, putative [Arabidopsis thaliana]
Cit.30042.1.S1_at	3,54	up	DN959492	NP_564588.2	1E-52	MATE efflux family protein [Arabidopsis thaliana]

Cit.17291.1.S1_at	15,92	up	CX302017	AAF60951.1	3E-77	O-methyltransferase [Populus balsamifera subsp. trichocarpa x Populus deltoides]
Cit.25554.1.S1_at	16,24	up	CX669376	AAK50769.1	1E-63	polygalacturonase [Pisum sativum]
Cit.8718.1.S1_s_at	19,42	up	CX670983	CAA50561.1	2E-99	catechol O-methyltransferase [Nicotiana tabacum]
Cit.20071.1.S1_s_at	20,93	up	CX666732	AAP33475.1	4E-90	polygalacturonase-like protein [Fragaria x ananassa]
Cit.12490.1.S1_s_at	22,09	up	CX044619	AAM20001.1	1E-134	putative polygalacturonase PG1 [Arabidopsis thaliana]
Cit.9706.1.S1_s_at	23,17	up	CV886686	CAA03908.1	1E-145	beta-1,3-glucanase [Citrus sinensis]
Cit.10437.1.S1_s_at	33,82	up	CX665316	AAP33475.1	1E-144	polygalacturonase-like protein [Fragaria x ananassa]
Cit.21559.1.S1_s_at	34,76	up	CX051882	AAP33475.1	4E-33	polygalacturonase-like protein [Fragaria x ananassa]
Cit.2392.1.S1_at	42,38	up	CF831790	AAB65155.1	0.0	acidic cellulase [Citrus sinensis]

Disease resistance, defence and stress response

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.5743.1.S1_s_at	3,01	up	CX643231	AAD11484.1	3E-44	peroxidase [Glycine max]
Cit.17781.1.S1_at	3,02	up	DN799310	CAA55862.1	1E-29	ubiquinol--cytochrome c reductase [Solanum tuberosum]
Cit.11854.1.S1_at	3,04	up	CX046093	CAA71492.1	5E-98	peroxidase [Spinacia oleracea]
Cit.25478.1.S1_at	3,05	up	CX666877	AAP03018.1	1E-100	4-coumarate-CoA ligase-like protein [Arabidopsis thaliana]
Cit.2538.1.S1_at	3,05	up	CF837686	NP_181526.1	2E-40	peroxisomal membrane protein (PMP36) [Arabidopsis thaliana]
Cit.17961.1.S1_s_at	3,05	up	DN798478	BAB16427.1	4E-12	NtEIG-E80 [Nicotiana tabacum]
Cit.20982.1.S1_s_at	3,06	up	DN795079	BAB09573.1	8E-04	AAA-type ATPase-like protein [Arabidopsis thaliana]
Cit.19053.1.S1_at	3,07	up	CX302166	AAV34889.1	9E-26	osmotin-like [Theobroma cacao]
Cit.26764.1.S1_at	3,08	up	CV713176	BAD29345.1	7E-19	zinc finger protein-like [Oryza sativa (japonica cultivar-group)]
Cit.2718.1.S1_s_at	3,08	up	CK939764	AAC61283.1	8E-81	22 kDa peroxisomal membrane protein [Arabidopsis thaliana]
Cit.37302.1.S1_at	3,10	up	BQ624679	NP_189443.2	5E-61	leucine-rich repeat transmembrane protein kinase, putative [Arabidopsis thaliana]
Cit.31341.1.S1_at	3,10	up	CX297758	BAC21263.1	2E-51	glutathione S-transferase [Cucurbita maxima]
Cit.10645.1.S1_at	3,10	up	CX664652	AAN28853.1	2E-32	At4g27500/F27G19_100 [Arabidopsis thaliana]
Cit.6048.1.S1_s_at	3,11	up	CV715327	AAF61863.1	3E-40	DNA-binding protein 3 [Nicotiana tabacum]
Cit.17765.1.S1_x_at	3,12	up	CX304476	CAA48027.1	7E-05	Eli3-1 [Arabidopsis thaliana]
Cit.6998.1.S1_at	3,12	up	CX043364	AAR13301.1	3E-35	anthocyanin acyltransferase [Phaseolus vulgaris]
Cit.9587.1.S1_at	3,13	up	CX293561	AAG30140.1	7E-82	glutathione S-transferase [Arabidopsis thaliana]
Cit.2538.1.S1_s_at	3,13	up	CF837686	NP_181526.1	2E-40	peroxisomal membrane protein (PMP36) [Arabidopsis thaliana]
Cit.19486.1.S1_at	3,13	up	CK933061	NP_849378.1	1E-44	peroxisomal membrane protein-related [Arabidopsis thaliana]
Cit.5309.1.S1_at	3,15	up	CV717349	NP_199935.2	7E-86	ubiquinol-cytochrome C chaperone family protein [Arabidopsis thaliana]
Cit.12475.1.S1_at	3,16	up	CX640275	CAA54613.1	3E-92	UTP-glucose glucosyltransferase [Manihot esculenta]
Cit.31138.1.S1_at	3,17	up	CN184779	NP_200184.1	2E-73	harpin-induced family protein / HIN1 family protein / harpin-responsive family protein [Arabidopsis thaliana]
Cit.17228.1.S1_at	3,19	up	CV715877	AAB36652.1	1E-101	immediate-early salicylate-induced glucosyltransferase
Cit.11856.1.S1_at	3,19	up	AY243478	AAO72741.1	0.0	allene oxide synthase [Citrus sinensis]
Cit.25974.1.S1_at	3,20	up	CF832469	NP_177466.1	7E-22	nascent polypeptide-associated complex (NAC) domain-containing protein [Arabidopsis thaliana]
Cit.17762.1.S1_at	3,22	up	CN183091	BAB84352.1	3E-44	lipoxygenase [Citrus jambhiri]
Cit.32625.1.S1_s_at	3,22	up	CX292843	CAA58733.1	4E-07	PAR-1a [Nicotiana tabacum]
Cit.27321.1.S1_at	3,22	up	CX070547	AAN28917.1	3E-61	At4g25340/T30C3_20 [Arabidopsis thaliana]
Cit.28036.1.S1_s_at	3,22	up	CX672359	BAB01326.1	4E-94	receptor-like kinase [Arabidopsis thaliana]
Cit.17265.1.S1_at	3,24	up	CK937355	BAA92155.1	2E-19	glycine-rich protein [Citrus unshiu]
Cit.10638.1.S1_at	3,26	up	CV884532	NP_564149.1	1E-106	20S proteasome beta subunit C1 (PBC1) (PRCT) [Arabidopsis thaliana]
Cit.37306.1.S1_at	3,27	up	BQ624718	NP_193956.2	1E-08	zinc finger (C3HC4-type RING finger) protein family [Arabidopsis thaliana]
Cit.4177.1.S1_at	3,30	up	CX047099	AAO50725.1	1E-102	putative lipase [Arabidopsis thaliana]
Cit.11356.1.S1_s_at	3,31	up	CF837067	AAM73656.1	4E-80	AER [Nicotiana tabacum]
Cit.13716.1.S1_s_at	3,31	up	DN625547	CAB42912.1	2E-85	putative cold acclimation protein [Arabidopsis thaliana]
Cit.304.1.S1_s_at	3,31	up	BQ623782	CAE53882.1	1E-142	aquaporin [Ricinus communis]
Cit.6937.1.S1_at	3,33	up	CX546610	AAL25542.1	4E-12	At1g73800/F25P22_22 [Arabidopsis thaliana]
Cit.16551.1.S1_at	3,34	up	CV705388	DAA00872.1	3E-57	TPA: PDR4 ABC transporter [Arabidopsis thaliana]
Cit.15757.1.S1_at	3,35	up	CX077288	NP_182100.2	5E-08	BON1-associated protein (BAP1)-related [Arabidopsis thaliana]
Cit.6660.1.S1_at	3,37	up	CX306249	CAC18730.1	1E-100	NADH glutamate dehydrogenase [Vitis vinifera]
Cit.5206.1.S1_at	3,38	up	CX545682	NP_200956.1	6E-72	leucine-rich repeat transmembrane protein kinase, putative [Arabidopsis thaliana]
Cit.10716.1.S1_at	3,38	up	CV713082	NP_568984.1	4E-43	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein [Arabidopsis thaliana]
Cit.1820.1.S1_at	3,40	up	DN797852	BAA21921.1	9E-37	ZPT2-12 [Petunia x hybrida]
Cit.26982.1.S1_at	3,42	up	CV716390	NP_189066.1	1E-92	leucine-rich repeat transmembrane protein kinase, putative [Arabidopsis thaliana]
Cit.425.1.S1_s_at	3,44	up	CX047220	CAA80963.1	4E-46	blue copper protein [Pisum sativum]
Cit.7331.1.S1_at	3,44	up	CK665301	AAS80149.1	2E-49	ACT11D09.3 [Cucumis melo]
Cit.3236.1.S1_at	3,46	up	CK939355	NP_568568.1	5E-39	calcium-binding EF hand family protein [Arabidopsis thaliana]
Cit.5664.1.S1_at	3,47	up	CX671291	AAT28308.1	1E-67	leucine-rich repeat receptor-like protein kinase [Pyrus pyrifolia]
Cit.12803.1.S1_s_at	3,49	up	DN625595	BAB02040.1	1E-102	serine/threonine kinase [Arabidopsis thaliana]
Cit.5206.1.S1_s_at	3,52	up	CX545682	NP_200956.1	6E-72	leucine-rich repeat transmembrane protein kinase, putative [Arabidopsis thaliana]
Cit.30042.1.S1_at	3,54	up	DN959492	NP_564588.2	1E-52	MATE efflux family protein [Arabidopsis thaliana]

Cit.17919.1.S1_s_at	4,99	up	DN795283	Q93ZH0	1E-106	LysM-domain GPI-anchored protein 1 precursor gb|AAL09782.1| At1g21880/T26F17_5 [Arabidopsis thaliana]
Cit.31497.1.S1_at	5,03	up	DN958192	CAB45652.1	2E-61	nodulin26-like intrinsic protein [Pisum sativum]
Cit.326.1.S1_s_at	5,11	up	CV885111	AAO39008.1	1E-143	plasma intrinsic protein 2,2 [Juglans regia]
Cit.8908.1.S1_s_at	5,19	up	CX674698	AAC39480.1	3E-92	aquaporin [Vernicia fordii]
Cit.233.1.S1_at	5,19	up	CF828233	Q39967	3E-21	Major latex allergen Hev b 5
Cit.31136.1.S1_at	5,21	up	CX299481	BAC42063.1	1E-114	putative cysteine proteinase [Arabidopsis thaliana]
Cit.5228.1.S1_s_at	5,22	up	DN622543	AAK59558.1	1E-31	putative receptor-protein kinase [Arabidopsis thaliana]
Cit.12789.1.S1_s_at	5,23	up	CV717653	AAS58497.1	2E-71	aminopeptidase P short isoform [Arabidopsis thaliana]
Cit.19571.1.S1_s_at	5,23	up	CK935883	NP_176113.1	9E-59	disease resistance-responsive protein-related / dirigent protein-related [Arabidopsis thaliana]
Cit.2027.1.S1_s_at	5,23	up	DN617689	AAD02832.1	1E-55	raffinose synthase [Cucumis sativus]
Cit.30574.1.S1_s_at	5,23	up	CX671094	AAV67891.1	2E-67	betaine-aldehyde dehydrogenase [Chorispora bungeana]
Cit.30441.1.S1_at	5,25	up	CX635152	AAD10032.1	1E-55	translationally controlled tumor protein [Hevea brasiliensis]
Cit.11327.1.S1_s_at	5,29	up	CX071464	CAG14984.1	9E-37	putative lipid transfer protein GPI-anchored [Cicer arietinum]
Cit.30406.1.S1_at	5,33	up	CK934189	NP_567717.1	5E-31	immunophilin-related / FKBP-type peptidyl-prolyl cis-trans isomerase-related [Arabidopsis thaliana]
Cit.14683.1.S1_s_at	5,40	up	CN181971	NP_197731.1	7E-71	disease resistance family protein / LRR family protein [Arabidopsis thaliana]
Cit.3628.1.S1_at	5,43	up	CX077465	AAF14041.1	6E-54	putative laccase [Arabidopsis thaliana]
Cit.15490.1.S1_at	5,43	up	CX674940	AAF33670.1	2E-67	cyclic nucleotide-gated calmodulin-binding ion channel [Nicotiana tabacum]
Cit.26653.1.S1_at	5,43	up	CX043514	AAR28378.1	2E-34	EIX receptor 2 [Lycopersicon esculentum]
Cit.8203.1.S1_x_at	5,51	up	CB290246	AAQ92310.1	4E-74	COR15 [Citrus clementina x Citrus reticulata]
Cit.9825.1.S1_at	5,57	up	DN620336	BAA89230.1	1E-47	wts2L [Citrullus lanatus]
Cit.7906.1.S1_at	5,57	up	CX636220	AAM61746.1	6E-65	cytochrome P450 monooxygenase [Arabidopsis thaliana]
Cit.18275.1.S1_at	5,65	up	CX302100	NP_196694.1	2E-59	monooxygenase family protein [Arabidopsis thaliana]
Cit.10407.1.S1_s_at	5,65	up	CF836847	AAR23816.2	1E-107	betaine-aldehyde dehydrogenase [Gossypium hirsutum]
Cit.3911.1.S1_s_at	5,77	up	CX673500	AAP83137.1	3E-98	lipoxygenase [Nicotiana attenuata]
Cit.580.1.S1_x_at	5,78	up	CX637285	CAA41437.1	4E-57	glutathione S-transferase [Arabidopsis thaliana]
Cit.8558.1.S1_s_at	5,83	up	CB290914	CAA83565.1	1E-160	INO1 [Citrus x paradisi]
Cit.11113.1.S1_s_at	5,88	up	CF833984	NP_192839.1	1E-107	nucleoside diphosphate kinase 3, mitochondrial (NDK3) [Arabidopsis thaliana]
Cit.1990.1.S1_s_at	5,89	up	CX043748	AAM14191.1	6E-36	putative chaperonin CPN10 protein [Arabidopsis thaliana]
Cit.13658.1.S1_at	5,91	up	CK740163	AAC42249.1	1E-118	putative aquaporin (tonoplast intrinsic protein) [Arabidopsis thaliana]
Cit.11691.1.S1_at	6,13	up	CF832883	CAB42906.1	3E-48	calmodulin-like protein [Arabidopsis thaliana]
Cit.12743.1.S1_at	6,18	up	DN625518	AAG16758.1	1E-64	putative glutathione S-transferase T3 [Lycopersicon esculentum]
Cit.4457.1.S1_s_at	6,19	up	CD574780	AAP03023.1	3E-82	acyl-activating enzyme 12 [Arabidopsis thaliana]
Cit.5847.1.S1_a_at	6,22	up	CN184532	AAF07830.1	5E-54	putative SCO1 protein [Arabidopsis thaliana]
Cit.9584.1.S1_x_at	6,23	up	DR403286	AAG30140.1	9E-83	glutathione S-transferase [Arabidopsis thaliana]
Cit.9300.1.S1_s_at	6,25	up	CB290828	BAA74434.1	3E-69	similar to hsr203J [Lycopersicon esculentum]
Cit.1966.1.S1_s_at	6,27	up	CX665191	CAC14058.1	1E-126	acridone synthase [Ruta graveolens]
Cit.6334.1.S1_at	6,32	up	CV715984	BAB10434.1	5E-99	ABC transporter-like protein [Arabidopsis thaliana]
Cit.9686.1.S1_x_at	6,46	up	CX303266	NP_563648.1	1E-81	cathepsin B-like cysteine protease, putative [Arabidopsis thaliana]
Cit.23259.1.S1_s_at	6,69	up	CX676279	AAB95118.1	3E-88	pathogenesis-related group 5 protein [Brassica rapa]
Cit.753.1.S1_x_at	6,97	up	CF835337	CAA41437.1	1E-59	pathogenesis-related protein 4A [Nicotiana tabacum]
Cit.18077.1.S1_at	7,26	up	DN798370	AAM61225.1	3E-60	putative NADH dehydrogenase (ubiquinone oxidoreductase) [A. thaliana]
Cit.15593.1.S1_s_at	7,29	up	CX641508	BAB02603.1	6E-14	leucoanthocyanidin dioxygenase-like protein [Arabidopsis thaliana]
Cit.19727.1.S1_x_at	7,35	up	CK937930	BAB40143.1	4E-31	plasma membrane intrinsic protein 2-2 [Pyrus communis]
Cit.19552.1.S1_s_at	7,39	up	CK936083	AAG22740.1	3E-39	allergenic isoflavone reductase-like protein Bet v 6.0102 [Betula pendula]
Cit.2993.1.S1_at	7,56	up	CX663481	AAK62343.2	9E-64	elicitor-inducible cytochrome P450 [Nicotiana tabacum]
Cit.11230.1.S1_at	7,76	up	CK936665	BAB16426.1	4E-78	elicitor inducible gene product EIG-I24 [Nicotiana tabacum]
Cit.29898.1.S1_at	7,77	up	CK937073	NP_193829.2	7E-30	basic helix-loop-helix (bHLH) family protein [Arabidopsis thaliana]
Cit.7001.1.S1_at	7,98	up	CF418865	NP_174753.1	6E-35	oxidoreductase, 2OG-Fe(II) oxygenase family protein [Arabidopsis thaliana]
Cit.12788.1.S1_at	8,15	up	CX291626	AAS58497.1	1E-110	aminopeptidase P short isoform [Arabidopsis thaliana]
Cit.18296.1.S1_at	8,17	up	CF507396	NP_029428.1	4E-19	predicted GPI-anchored protein [Arabidopsis thaliana]
Cit.4661.1.S1_s_at	8,23	up	CV713066	AAT68601.1	1E-118	benzoyl coenzyme A: benzyl alcohol benzoyl transferase [Petunia x hybrida]
Cit.14913.1.S1_s_at	8,32	up	CX043703	CAA77656.1	6E-73	acidic chitinase III [Nicotiana tabacum]
Cit.14757.1.S1_at	8,59	up	CK702467	NP_566082.1	6E-37	calcium-binding protein, putative [Arabidopsis thaliana]
Cit.29373.1.S1_s_at	9,09	up	CK934765	AAG38517.1	1E-103	miraculin-like protein [Citrus x paradisi]
Cit.21654.1.S1_s_at	9,17	up	CX299958	AAC35981.1	5E-27	chitinase CHI1 [Citrus sinensis]
Cit.27793.1.S1_at	9,21	up	CX675562	AAD20706.1	1E-51	putative disease resistance protein [Arabidopsis thaliana]
Cit.2113.1.S1_at	9,24	up	CK934775	NP_176113.1	2E-59	disease resistance-responsive protein-related / dirigent protein-related [Arabidopsis thaliana]
Cit.8206.1.S1_s_at	9,55	up	CF504694	BAB84352.1	1E-101	lipoxygenase [Citrus jambhiri]
Cit.4763.1.S1_at	9,57	up	CX050343	AAL85086.1	1E-93	putative inorganic pyrophosphatase [Arabidopsis thaliana]
Cit.10594.1.S1_at	9,77	up	DN624837	AAC35981.1	1E-78	chitinase CHI1 [Citrus sinensis]
Cit.15242.1.S1_at	10,38	up	CX292066	AAC35981.1	1E-101	chitinase CHI1 [Citrus sinensis]
Cit.28591.1.S1_at	10,90	up	CV710110	AAN31815.1	5E-36	putative nodulin [Arabidopsis thaliana]

Cit.40341.1.S1_x_at	11,00	up	DR405740	Q39967	1E-20	Major latex allergen Hev b 5
Cit.21128.1.S1_at	11,41	up	DN799150	BAB02603.1	4E-27	leucoanthocyanidin dioxygenase-like protein [Arabidopsis thaliana]
Cit.376.1.S1_x_at	11,74	up	CX666561	Q39967	5E-22	Major latex allergen Hev b 5
Cit.16900.1.S1_x_at	11,96	up	CB610530	Q39967	6E-21	Major latex allergen Hev b 5
Cit.9301.1.S1_s_at	12,26	up	CN190145	BAD11070.1	1E-102	HSR203J like protein [Capsicum chinense]
Cit.100.1.S1_x_at	12,34	up	CK934397	Q39967	3E-21	Major latex allergen Hev b 5
Cit.26307.1.S1_s_at	12,48	up	CV884507	CAH03799.1	4E-26	lipid transfer protein [Citrus sinensis]
Cit.31377.1.S1_at	13,37	up	DN619712	AAB81668.1	8E-81	NAM (no apical meristem)-like protein [Arabidopsis thaliana]
Cit.1200.1.S1_at	13,56	up	CX292655	AAM21199.1	7E-90	pathogenesis-related protein 5-1 [Helianthus annuus]
Cit.721.1.S1_x_at	14,64	up	CB290748	Q39967	5E-22	Major latex allergen Hev b 5
Cit.14939.1.S1_at	18,09	up	CV718780	XP_476016.1	1E-102	putative hypersensitive-induced response protein
Cit.18517.1.S1_at	20,18	up	CX293755	AAO27256.1	4E-39	putative NADH-dehydrogenase [Pisum sativum]
Cit.19313.1.S1_at	21,42	up	CX308038	EAL71975.1	2E-04	putative protein serine/threonine kinase [Dictyostelium discoideum]
Cit.5769.1.S1_at	35,44	up	CN189092	AAK62346.1	3E-57	elicitor-inducible cytochrome P450 [Nicotiana tabacum]
Cit.15404.1.S1_at	53,39	up	CF653559	AAK30143.1	2E-58	pathogenesis-related protein PR-1 precursor [Capsicum annuum]
Ethylene synthesis and signalling						
Probe Set ID	**FC**	**Reg**	**Rep Public ID**	**Protein ID**	**e-value**	**Target Description**
Cit.18154.1.S1_at	4,15	up	CX298890	AAB88878.1	1E-52	ethylene-forming-enzyme-like dioxygenase [Prunus armeniaca]
Cit.4491.1.S1_at	8,05	up	CX643923	CAB60722.1	0.0	ACC synthase [Citrus sinensis]
Cit.30535.1.S1_s_at	10,23	up	CB322167	AAG49361.1	1E-101	ACC oxidase [Citrus sinensis]
Cit.21723.1.S1_s_at	11,18	up	CX305211	AAG49361.1	2E-31	ACC oxidase [Citrus sinensis]
Putative Transcriptional Factors						
Probe Set ID	**FC**	**Reg**	**Rep Public ID**	**Protein ID**	**e-value**	**Target Description**
Cit.39642.1.S1_at	3,73	up	DN799348	CAI38917.1	5E-33	putative WRKY transcription factor 10 [Nicotiana tabacum]
Cit.22353.1.S1_s_at	4,48	up	CF831078	NP_178143.1	5E-79	DNA binding protein, putative [Arabidopsis thaliana]
Cit.8822.1.S1_s_at	6,14	up	CF835408	AAN13013.1	6E-90	putative chloroplast nucleoid DNA-binding protein [Arabidopsis thaliana]
Cit.5203.1.S1_at	7,06	up	CX676151	AAD39676.1	1E-76	F9L1.43 [Arabidopsis thaliana]
Cit.8823.1.S1_s_at	9,17	up	CN182354	AAN13013.1	1E-113	putative chloroplast nucleoid DNA-binding protein [Arabidopsis thaliana]
Terpene, GA and volatile synthesis						
Probe Set ID	**FC**	**Reg**	**Rep Public ID**	**Protein ID**	**e-value**	**Target Description**
Cit.9990.1.S1_x_at	3,07	up	CX663607	CAA46273.1	1E-88	GA [Pisum sativum]
Cit.1441.1.S1_at	3,38	up	CN191360	BAB01067.1	8E-36	acetyltranferase-like protein [Arabidopsis thaliana]
Cit.36909.1.S1_s_at	3,54	up	CD573732	AAQ20892.1	2E-94	10-hydroxygeraniol oxidoreductase [Camptotheca acuminata]
Cit.10053.1.S1_at	3,69	up	CX302245	CAA09804.2	1E-133	1-deoxyxylulose 5-phosphate synthase [Catharanthus roseus]
Cit.16670.1.S1_at	4,00	up	CX669501	BAA78047.1	5E-70	GGPP synthase [Daucus carota]
Cit.10826.1.S1_s_at	4,01	up	CX665594	BAA78047.1	1E-69	GGPP synthase [Daucus carota]
Cit.17115.1.S1_at	4,16	up	CF834454	T05262	2E-12	farnesyl-diphosphate farnesyltransferase (EC 2.5.1.21) - soybean
Cit.17714.1.S1_at	4,83	up	CF828804	AAG21984.1	5E-12	LYTB-like protein precursor [Adonis palaestina]
Cit.9942.1.S1_x_at	5,03	up	CB291954	AAQ20892.1	1E-107	10-hydroxygeraniol oxidoreductase [Camptotheca acuminata]
Cit.2586.1.S1_s_at	5,09	up	CX663894	NP_173852.1	2E-36	transferase family protein [Arabidopsis thaliana]
Cit.27992.1.S1_s_at	5,32	up	CX671596	BAB01067.1	2E-29	acetyltranferase-like protein [Arabidopsis thaliana]
Cit.12119.1.S1_s_at	5,90	up	BQ624758	AAO23063.1	4E-80	ent-kaurenoic acid oxidase [Pisum sativum]
Cit.29411.1.S1_s_at	6,42	up	CF418090	NP_189233.1	8E-37	transferase family protein [Arabidopsis thaliana]
Cit.17898.1.S1_x_at	8,21	up	DN623336	Q43246	4E-04	Cytochrome P450 88A1 (Dwarf3 protein) pir||T02263 cytochrome P450 DWARF3 - maize
Cit.9944.1.S1_x_at	10,81	up	CB291627	AAQ20892.1	1E-125	10-hydroxygeraniol oxidoreductase [Camptotheca acuminata]
Vesicle trafficking						
Probe Set ID	**FC**	**Reg**	**Rep Public ID**	**Protein ID**	**e-value**	**Target Description**
Cit.12958.1.S1_s_at	3,34	up	CK935417	CAA98178.1	7E-92	RAB11B [Lotus corniculatus var. japonicus]
Cit.16671.1.S1_at	5,49	up	CV719481	CAD78064.1	8E-41	knolle [Antirrhinum majus]
Down Regulated						
Probe Set ID	**FC**	**Reg**	**Rep Public ID**	**Protein ID**	**e-value**	**Target Description**
Cit.26611.1.S1_s_at	5,07	down	CN191278	NP_201058.1	1E-39	expressed protein [Arabidopsis thaliana]
Cit.29183.1.S1_at	5,07	down	CX643202	BAB01067.1	6E-25	acetyltranferase-like protein [Arabidopsis thaliana]
Cit.13667.1.S1_s_at	5,09	down	CN187977	AAO12870.1	1E-15	wound induced protein-like [Vitis vinifera]
Cit.26611.1.S1_at	5,11	down	CN191278	NP_201058.1	1E-39	expressed protein [Arabidopsis thaliana]
Cit.35331.1.S1_at	5,13	down	CK938655	NP_200685.1	2E-20	dehydrodolichyl diphosphate synthase, putative / DEDOL-PP synthase, putative [Arabidopsis thaliana]
Cit.1779.1.S1_at	5,22	down	CN185436	AAN74634.1	7E-59	heat shock protein [Pisum sativum]
Cit.4160.1.S1_at	5,24	down	CV709551	Q7G8V2	7E-09	Two-component response regulator ARR15
Cit.30664.1.S1_x_at	5,27	down	CX307271	CAE03090.2	6E-10	OSJNBa0017B10.5 [Oryza sativa (japonica cultivar-group)]
Cit.3195.1.S1_at	5,29	down	CB291604	NP_564416.1	9E-12	expressed protein [Arabidopsis thaliana]
Cit.5673.1.S1_at	5,35	down	CX674077	AAP83139.1	1E-55	N-acylethanolamine amidohydrolase [Arabidopsis thaliana]
Cit.8714.1.S1_s_at	5,35	down	CX049693	AAO49652.1	3E-64	photosystem I-N subunit [Phaseolus vulgaris]

Cit.33051.1.S1_s_at	5,38	down	CX296814	AAD27882.2	2E-72	chlorophyll a/b-binding protein CP24 precursor [Vigna radiata]
Cit.1117.1.S1_s_at	5,68	down	CX671580	NP_175963.1	8E-59	photosystem I reaction center subunit V, chloroplast, putative / PSI-G, putative (PSAG) [Arabidopsis thaliana]
Cit.3693.1.S1_at	5,69	down	DN797794	CAC10358.1	9E-58	protein phosphatase 2C [Nicotiana tabacum] emb|CAC84141.2| protein phosphatase 2C [Nicotiana tabacum]
Cit.30664.1.S1_at	5,72	down	CX307271	CAE03090.2	6E-10	OSJNBa0017B10.5 [Oryza sativa (japonica cultivar-group)]
Cit.32002.1.S1_at	5,72	down	CX287685	BAB01805.1	3E-50	unnamed protein product [Arabidopsis thaliana]
Cit.14749.1.S1_at	5,84	down	CX671813	BAB86920.1	3E-98	glucosyltransferase-2 [Vigna angularis]
Cit.14324.1.S1_s_at	5,88	down	CD576701	AAN12962.1	5E-31	unknown protein [Arabidopsis thaliana]
Cit.30576.1.S1_s_at	5,89	down	CX292882	AAM51559.1	8E-49	putative guanylate cyclase [Arabidopsis thaliana]
Cit.28980.1.S1_s_at	6,04	down	CX636045	AAQ21125.1	3E-50	pectinesterase [Fragaria x ananassa]
Cit.32940.1.S1_at	6,08	down	CX295749	AAR28998.1	4E-74	CMV 1a interacting protein 1 [Nicotiana tabacum]
Cit.34811.1.S1_at	6,09	down	C22251	NP_179257.1	1E-39	nodulin family protein [Arabidopsis thaliana]
Cit.33199.1.S1_at	6,22	down	CX298367	BAC42315.1	2E-78	unknown protein [Arabidopsis thaliana]
Cit.8928.1.S1_s_at	6,32	down	CD575272	P29302	2E-89	Photosystem I reaction center subunit II, chloroplast precursor (Photosystem I 20 kDa subunit) (PSI-D)
Cit.18045.1.S1_s_at	6,43	down	CN185220	AAM63508.1	6E-25	transcription factor TINY, putative [Arabidopsis thaliana]
Cit.9106.1.S1_s_at	6,44	down	CF837583	CAA45701.1	1E-13	3 33 kDa polypeptide of water-oxidizing complex of photosystem II [Nicotiana tabacum]
Cit.40420.1.S1_at	6,58	down	CK939963	AAQ88400.1	1E-55	CaCBF1B [Capsicum annuum]
Cit.15018.1.S1_at	6,59	down	CX300360	AAM63015.1	4E-21	probable wound-induced protein [Arabidopsis thaliana]
Cit.39509.1.S1_s_at	6,90	down	DN795213	AAP37804.1	2E-53	At2g15890 [Arabidopsis thaliana]
Cit.18537.1.S1_at	6,95	down	CX294219	AAP37804.1	2E-16	At2g15890 [Arabidopsis thaliana]
Cit.4608.1.S1_at	7,03	down	CB293889	AAF79590.1	2E-32	F28C11.18 [Arabidopsis thaliana]
Cit.7494.1.S1_at	7,16	down	CX671518	BAC43539.1	9E-77	unknown protein [Arabidopsis thaliana]
Cit.16722.1.S1_at	7,30	down	CX074966	BAB86890.1	1E-106	syringolide-induced protein 19-1-5 [Glycine max]
Cit.5237.1.S1_s_at	7,36	down	CX070296	CAB65284.1	1E-20	putative wound-induced protein [Medicago sativa subsp. x varia]
Cit.35313.1.S1_s_at	7,73	down	CK938925	AAL06483.1	4E-65	At2g20670/F23N11.1 [Arabidopsis thaliana]
Cit.29721.1.S1_at	8,82	down	CV715902	CAB65284.1	1E-20	putative wound-induced protein [Medicago sativa subsp. x varia]
Cit.11435.1.S1_s_at	8,83	down	DN619965	NP_566623.1	9E-69	oxidoreductase, 2OG-Fe(II) oxygenase family protein [Arabidopsis thaliana]
Cit.17203.1.S1_s_at	9,01	down	CX636120	CAB75430.1	8E-61	putative 16kDa membrane protein [Nicotiana tabacum]
Cit.14413.1.S1_at	9,79	down	CK939761	NP_197105.1	2E-55	steroid 5-alpha-reductase family protein [Arabidopsis thaliana]
Cit.30525.1.S1_at	11,31	down	CX290693	AAF09487.1	1E-55	short chain alcohol dehydrogenase [Arabidopsis thaliana]
Cit.6333.1.S1_at	11,83	down	CX673626	AAO64802.1	2E-76	At4g17030 [Arabidopsis thaliana]

Unknown/Hypothetical

Probe Set ID	***FC***	***Reg***	***Rep Public ID***	***Protein ID***	***e-value***	***Target Description***
Cit.13871.1.S1_at	4,98	up	CX670035	BAB11323.1	1E-102	4-nitrophenylphosphatase-like protein [Arabidopsis thaliana]
Cit.14610.1.S1_s_at	3,30	up	DT214449	NP_566139.1	1E-127	5 -AMP-activated protein kinase beta-1 subunit-related [Arabidopsis thaliana]
Cit.1939.1.S1_s_at	3,24	up	CN189059	AAC26045.1	1E-107	aconitase-iron regulated protein 1 [Citrus limon]
Cit.13708.1.S1_at	3,21	up	CV887255	BAA01181.1	1E-112	adenylate kinase-b [Oryza sativa]
Cit.15251.1.S1_at	3,75	up	CX543471	AAU03365.1	2E-48	alpha/beta fold family protein [Lycopersicon esculentum]
Cit.38868.1.S1_at	3,41	up	CK740055	NP_175442.2	1E-51	armadillo/beta-catenin repeat family protein [Arabidopsis thaliana]
Cit.3891.1.S1_at	3,11	up	CV715852	NP_191315.1	6E-94	aspartate/glutamate/uridylate kinase family protein [Arabidopsis thaliana]
Cit.13780.1.S1_at	3,14	up	CV714662	BAC87890.1	7E-26	aspartic acid-rich protein aspolin2 [Theragra chalcogramma]
Cit.23577.1.S1_s_at	3,01	up	DN621135	BAC16371.1	1E-47	aspartic proteinase 5 [Glycine max]
Cit.9688.1.S1_x_at	5,32	up	CF833118	AAL16267.1	1E-146	At1g02300/T6A9_10 [Arabidopsis thaliana]
Cit.14250.1.S1_at	4,59	up	CV713259	AAL16234.1	2E-84	At1g04040/F21M11_2 [Arabidopsis thaliana]
Cit.7545.1.S1_at	3,66	up	CX043458	AAP68220.1	1E-100	At1g11090 [Arabidopsis thaliana]
Cit.1492.1.S1_at	4,00	up	CV708784	AAM51596.1	1E-101	At1g11930/F12F1_20 [Arabidopsis thaliana]
Cit.14168.1.S1_at	4,15	up	CX666171	AAU15136.1	1E-110	At1g22440 [Arabidopsis thaliana]
Cit.15469.1.S1_at	3,02	up	CX670154	AAQ65113.1	9E-18	At1g23530 [Arabidopsis thaliana]
Cit.4137.1.S1_s_at	3,33	up	CF417094	AAO64790.1	1E-85	At1g50490 [Arabidopsis thaliana]
Cit.23843.1.S1_at	6,38	up	CV707277	AAP21270.1	7E-44	At1g60680 [Arabidopsis thaliana]
Cit.34156.1.S1_at	3,56	up	CX304790	AAO64854.1	1E-73	At1g64600 [Arabidopsis thaliana]
Cit.9453.1.S1_at	3,65	up	CX070985	AAS49083.1	4E-67	At1g79140 [Arabidopsis thaliana]
Cit.31406.1.S1_at	3,10	up	CF508506	AAM19795.1	1E-56	At2g04030/F3C11.14 [Arabidopsis thaliana]
Cit.16255.1.S1_at	3,68	up	CX308153	AAR25642.1	2E-58	At2g06030 [Arabidopsis thaliana]
Cit.26348.1.S1_at	3,30	up	CV886504	AAQ22668.1	3E-75	At2g17270 [Arabidopsis thaliana]
Cit.17542.1.S1_at	3,59	up	DN798632	AAP75797.1	2E-47	At2g32650 [Arabidopsis thaliana]
Cit.39558.1.S1_s_at	8,40	up	DN795545	AAN18146.1	3E-81	At2g38710/T6A23.9 [Arabidopsis thaliana]
Cit.10820.1.S1_at	8,96	up	CF420987	AAN18146.1	5E-95	At2g38710/T6A23.9 [Arabidopsis thaliana]
Cit.13371.1.S1_at	6,43	up	CX663975	AAL84929.1	1E-111	At2g46890/F19D11.17 [Arabidopsis thaliana]
Cit.2189.1.S1_at	3,28	up	CK935403	AAP88333.1	4E-46	At3g06890 [Arabidopsis thaliana]
Cit.14746.1.S1_at	3,70	up	CX077722	AAP21170.1	2E-69	At3g19170/MVI11_8 [Arabidopsis thaliana]
Cit.5065.1.S1_at	3,58	up	CX048432	AAM91356.1	2E-47	At3g19540/T31J18_4 [Arabidopsis thaliana]
Cit.5426.1.S1_s_at	6,16	up	CX545091	AAP21147.1	2E-95	At3g19970/MZE19_2 [Arabidopsis thaliana]

Cit.5425.1.S1_at	4,69	up	CV715605	AAP21147.1	3E-61	At3g19970/MZE19_2 [Arabidopsis thaliana]
Cit.22976.1.S1_at	3,85	up	CX045021	AAO42870.1	2E-20	At3g49180 [Arabidopsis thaliana]
Cit.10047.1.S1_s_at	3,25	up	CX663304	AAP13395.1	4E-79	At3g49940 [Arabidopsis thaliana]
Cit.16699.1.S1_at	3,13	up	CX289692	AAP21158.1	3E-31	At3g51740/T18N14_120 [Arabidopsis thaliana]
Cit.26191.1.S1_at	3,59	up	CN182492	AAL14384.1	5E-28	AT3g52500/F22O6_120 [Arabidopsis thaliana]
Cit.29954.1.S1_at	4,09	up	CX302645	AAP21218.1	1E-58	At3g55605 [Arabidopsis thaliana]
Cit.375.1.S1_x_at	5,56	up	CX640198	AAL09741.1	1E-100	AT4g05320/C17L7_240 [Arabidopsis thaliana]
Cit.24517.1.S1_s_at	3,11	up	CX546022	AAU05534.1	3E-28	At4g12320 [Arabidopsis thaliana]
Cit.24517.1.S1_at	6,39	up	CX546022	AAU05534.1	3E-28	At4g12320 [Arabidopsis thaliana]
Cit.15136.1.S1_s_at	4,35	up	CK933002	AAK49595.1	7E-29	AT4g15140/dl3615c [Arabidopsis thaliana]
Cit.14773.1.S1_at	3,19	up	CN181946	AAO64889.1	5E-66	At4g23180 [Arabidopsis thaliana]
Cit.38525.1.S1_at	3,41	up	CF509410	AAP68214.1	1E-75	At4g24530 [Arabidopsis thaliana]
Cit.23716.1.S1_at	3,65	up	CV705615	AAO11570.1	1E-35	At4g27830/T27E11_70 [Arabidopsis thaliana]
Cit.5904.1.S1_s_at	3,30	up	CX543169	AAK50083.1	1E-107	AT4g28450/F20O9_130 [Arabidopsis thaliana]
Cit.29003.1.S1_s_at	4,88	up	CX637270	AAR24736.1	4E-43	At4g32870 [Arabidopsis thaliana]
Cit.4713.1.S1_s_at	3,31	up	CV719316	AAN28791.1	1E-84	At4g38150/F20D10_270 [Arabidopsis thaliana]
Cit.31052.1.S1_at	8,45	up	DN959261	AAP88362.1	6E-32	At5g02050 [Arabidopsis thaliana]
Cit.23897.1.S1_at	4,38	up	CV708349	AAP21157.1	4E-08	At5g02970/F9G14_280 [Arabidopsis thaliana]
Cit.29560.1.S1_at	3,02	up	CX076115	AAQ65148.1	9E-26	At5g12240 [Arabidopsis thaliana]
Cit.11026.1.S1_at	3,51	up	CX299241	AAL87368.1	2E-42	AT5g13220/T31B5_40 [Arabidopsis thaliana]
Cit.7606.1.S1_at	4,32	up	CX076597	AAN28755.1	2E-72	At5g16250/T21H19_170 [Arabidopsis thaliana]
Cit.30392.1.S1_at	3,15	up	CX293675	AAN18155.1	1E-114	At5g17630/K10A8_110 [Arabidopsis thaliana]
Cit.5609.1.S1_at	3,26	up	CV884719	AAS76239.1	3E-52	At5g19340 [Arabidopsis thaliana]
Cit.4048.1.S1_at	3,82	up	CB293938	AAL79597.1	1E-106	AT5g21070/T10F18_100 [Arabidopsis thaliana]
Cit.27885.1.S1_at	3,48	up	CX047067	AAP37859.1	2E-22	At5g44040 [Arabidopsis thaliana]
Cit.27951.1.S1_at	3,60	up	CX051935	AAU90051.1	9E-92	At5g48370 [Arabidopsis thaliana]
Cit.15288.1.S1_at	3,36	up	CX666359	AAL38605.1	1E-148	AT5g50150/MPF21_17 [Arabidopsis thaliana]
Cit.6138.1.S1_at	3,40	up	CV716234	AAR24654.1	3E-85	At5g56120 [Arabidopsis thaliana]
Cit.10652.1.S1_s_at	3,20	up	CX073367	AAN46863.1	1E-129	At5g67360/K8K14_8 [Arabidopsis thaliana]
Cit.25089.1.S1_at	9,37	up	CX306331	BAD06417.1	1E-51	cytochrome P450 [Asparagus officinalis]
Cit.14829.1.S1_at	6,85	up	CF417791	NP_198460.1	1E-58	cytochrome P450 family protein [Arabidopsis thaliana]
Cit.3324.1.S1_s_at	5,49	up	CX665253	NP_176674.1	4E-83	cytochrome P450, putative [Arabidopsis thaliana]
Cit.3915.1.S1_s_at	7,57	up	CX669450	BAA96885.1	2E-61	cytochrome P450-like [Arabidopsis thaliana]
Cit.16570.1.S1_at	3,06	up	CV710623	NP_190419.3	4E-85	expressed protein [Arabidopsis thaliana]
Cit.14975.1.S1_at	3,08	up	CX043358	NP_188328.2	2E-68	expressed protein [Arabidopsis thaliana]
Cit.3459.1.S1_s_at	3,12	up	CX670919	NP_973597.1	5E-95	expressed protein [Arabidopsis thaliana]
Cit.38638.1.S1_at	3,40	up	CK665146	NP_567628.1	4E-21	expressed protein [Arabidopsis thaliana]
Cit.16059.1.S1_s_at	3,55	up	CV886656	NP_974897.1	2E-27	expressed protein [Arabidopsis thaliana]
Cit.16059.1.S1_at	3,87	up	CV886656	NP_974897.1	2E-27	expressed protein [Arabidopsis thaliana]
Cit.21914.1.S1_at	3,88	up	CB304982	NP_178036.1	4E-73	expressed protein [Arabidopsis thaliana]
Cit.29950.1.S1_at	3,26	up	CK933736	NP_193438.2	1E-126	expressed protein [Arabidopsis thaliana]
Cit.26207.1.S1_at	3,03	up	CN183097	NP_187893.1	1E-35	expressed protein [Arabidopsis thaliana]
Cit.31840.1.S1_at	3,78	up	AU300729	NP_568044.1	3E-59	expressed protein [Arabidopsis thaliana]
Cit.29756.1.S1_at	3,02	up	BQ623955	AAB67623.2	2E-75	expressed protein [Arabidopsis thaliana]
Cit.4595.1.S1_at	3,05	up	CX073826	AAD21478.1	7E-80	expressed protein [Arabidopsis thaliana]
Cit.6523.1.S1_at	4,19	up	CN191826	NP_565729.1	2E-62	expressed protein [Arabidopsis thaliana]
Cit.5026.1.S1_at	4,09	up	CF832851	NP_567804.1	5E-24	expressed protein [Arabidopsis thaliana]
Cit.20606.1.S1_at	3,85	up	CK740222	NP_563744.1	3E-11	expressed protein [Arabidopsis thaliana]
Cit.31347.1.S1_at	3,30	up	AU300349	NP_568647.1	9E-20	expressed protein [Arabidopsis thaliana]
Cit.17406.1.S1_at	3,97	up	CX302394	NP_200749.1	2E-54	expressed protein [Arabidopsis thaliana]
Cit.3707.1.S1_at	4,36	up	CD575636	NP_179959.1	2E-57	expressed protein [Arabidopsis thaliana]
Cit.8025.1.S1_at	3,15	up	CN187802	NP_565031.1	6E-33	expressed protein [Arabidopsis thaliana]
Cit.26097.1.S1_s_at	3,53	up	CF836028	NP_565353.1	3E-19	expressed protein [Arabidopsis thaliana]
Cit.19790.1.S1_at	3,52	up	CK937027	NP_565581.1	9E-40	expressed protein [Arabidopsis thaliana]
Cit.12435.1.S1_s_at	3,43	up	CV716616	NP_195298.1	9E-16	expressed protein [Arabidopsis thaliana]
Cit.12932.1.S1_s_at	4,75	up	CF653202	NP_194433.1	2E-89	expressed protein [Arabidopsis thaliana]
Cit.37215.1.S1_at	3,35	up	BQ623421	AAR01643.1	2E-14	expressed protein [Oryza sativa (japonica cultivar-group)]
Cit.6585.1.S1_at	3,62	up	CX298306	AAF79241.1	8E-93	F10B6.19 [Arabidopsis thaliana]
Cit.21680.1.S1_at	4,04	up	CX302677	AAF24810.1	3E-06	F12K11.5 [Arabidopsis thaliana]
Cit.6443.1.S1_s_at	3,87	up	CX665719	AAF06051.1	1E-115	F12P19.7 [Arabidopsis thaliana]
Cit.6443.1.S1_at	4,05	up	CX665719	AAF06051.1	1E-115	F12P19.7 [Arabidopsis thaliana]
Cit.16725.1.S1_at	5,18	up	CX545559	AAF79278.1	4E-51	F14D16.2 [Arabidopsis thaliana]

Cit.27127.1.S1_at	3,52	up	CV718254	AAF25983.1	2E-34	F15H18.10 [Arabidopsis thaliana]
Cit.6513.1.S1_at	3,61	up	CV885740	AAF79603.1	1E-92	F5M15.6 [Arabidopsis thaliana]
Cit.6513.1.S1_a_at	4,65	up	CV885740	AAF79603.1	1E-92	F5M15.6 [Arabidopsis thaliana]
Cit.16668.1.S1_at	3,76	up	CX076397	AAM61633.1	3E-95	Fe-superoxide dismutase precursor [Arabidopsis thaliana]
Cit.11257.1.S1_at	3,98	up	CN189266	NP_195463.1	1E-101	hydrolase, alpha/beta fold family protein [Arabidopsis thaliana]
Cit.11257.1.S1_s_at	3,37	up	CN189266	NP_195463.1	1E-101	hydrolase, alpha/beta fold family protein [Arabidopsis thaliana]
Cit.2468.1.S1_s_at	3,10	up	CX075599	AAC26971.1	1E-24	keratin [Canis familiaris]
Cit.14444.1.S1_at	3,42	up	CV714751	CAB79830.1	2E-78	kinase binding protein-like [Arabidopsis thaliana]
Cit.4243.1.S1_at	3,20	up	CF417204	CAA65750.1	3E-50	lamin [Arabidopsis thaliana]
Cit.37234.1.S1_at	3,27	up	BQ623655	NP_565446.1	9E-70	metaxin-related [Arabidopsis thaliana]
Cit.7615.1.S1_at	3,08	up	CX640823	CAA77094.1	1E-55	MtN30 [Medicago truncatula]
Cit.13005.1.S1_at	3,19	up	CD573819	CAA42622.1	3E-51	nsGRP-2 [Nicotiana sylvestris]
Cit.28626.1.S1_s_at	3,31	up	CV710534	XP_473433.1	1E-45	OSJNBa0010H02.7 [Oryza sativa (japonica cultivar-group)]
Cit.28500.1.S1_at	3,14	up	CV708594	XP_472855.1	5E-75	OSJNBa0022H21.5 [Oryza sativa (japonica cultivar-group)]
Cit.31344.1.S1_at	3,64	up	CX289198	CAD40428.2	2E-93	OSJNBa0035B13.1 [Oryza sativa (japonica cultivar-group)]
Cit.24643.1.S1_s_at	3,48	up	CX636483	CAE04893.2	1E-46	OSJNBa0042I15.15 [Oryza sativa (japonica cultivar-group)]
Cit.12867.1.S1_at	3,07	up	CV716142	XP_473643.1	6E-29	OSJNBa0064M23.16 [Oryza sativa (japonica cultivar-group)]
Cit.24116.1.S1_s_at	3,11	up	CD575057	CAE04865.2	8E-34	OSJNBa0086O06.13 [Oryza sativa (japonica cultivar-group)]
Cit.23820.1.S1_s_at	3,31	up	CV706990	XP_474425.1	2E-56	OSJNBa0088H09.21 [Oryza sativa (japonica cultivar-group)]
Cit.26116.1.S1_at	5,70	up	CF836772	CAD41584.3	9E-58	OSJNBa0088I22.16 [Oryza sativa (japonica cultivar-group)]
Cit.2063.1.S1_at	4,89	up	CX291650	CAE04594.2	1E-64	OSJNBb0006N15.11 [Oryza sativa (japonica cultivar-group)]
Cit.26058.1.S1_s_at	3,56	up	CF834912	CAE01640.2	7E-13	OSJNBb0021I10.2 [Oryza sativa (japonica cultivar-group)]
Cit.18676.1.S1_s_at	3,33	up	CX299074	NP_917409.1	2E-20	OSJNBb0024F06.14 [Oryza sativa (japonica cultivar-group)]
Cit.14547.1.S1_at	3,82	up	CX047256	CAE05718.2	8E-96	OSJNBb0065J09.14 [Oryza sativa (japonica cultivar-group)]
Cit.20085.1.S1_at	3,27	up	CX669369	XP_473130.1	2E-32	OSJNBb0065L13.6 [Oryza sativa (japonica cultivar-group)]
Cit.23327.1.S1_at	4,98	up	CX052699	CAD39837.2	4E-69	OSJNBb0072N21.3 [Oryza sativa (japonica cultivar-group)]
Cit.26190.1.S1_at	4,29	up	CN182392	NP_909223.1	1E-40	P0024G09.20 [Oryza sativa (japonica cultivar-group)]
Cit.26296.1.S1_at	3,13	up	CV884221	XP_463397.1	6E-18	P0025A05.27 [Oryza sativa (japonica cultivar-group)]
Cit.11860.1.S1_at	3,08	up	CV704704	XP_463480.1	1E-13	P0414E03.8 [Oryza sativa (japonica cultivar-group)]
Cit.17886.1.S1_s_at	3,39	up	CK939377	NP_915910.1	5E-05	P0468B07.6 [Oryza sativa (japonica cultivar-group)]
Cit.6525.1.S1_at	3,06	up	CN182493	NP_916089.1	2E-47	P0481E12.12 [Oryza sativa (japonica cultivar-group)]
Cit.32790.1.S1_at	3,19	up	CX294595	NP_915595.1	2E-19	P0489B03.24 [Oryza sativa (japonica cultivar-group)]
Cit.30668.1.S1_s_at	6,95	up	DN795568	NP_913447.1	4E-19	P0492F05.24 [Oryza sativa (japonica cultivar-group)]
Cit.7003.1.S1_at	5,57	up	CF835916	XP_323366.1	2E-04	predicted protein [Neurospora crassa]
Cit.22921.1.S1_at	3,85	up	CN192136	NP_188575.1	4E-16	pseudouridine synthase family protein [Arabidopsis thaliana]
Cit.28545.1.S1_s_at	3,00	up	CV709319	AAF98432.1	2E-35	purine permease [Arabidopsis thaliana]
Cit.32646.1.S1_at	4,44	up	CX293015	AAF26118.1	8E-75	putative 26S proteosome regulatory subunit [Arabidopsis thaliana]
Cit.13423.1.S1_s_at	3,45	up	CX071199	AAM14282.1	3E-80	putative 6,7-dimethyl-8-ribityllumazine synthase precursor [Arabidopsis thaliana]
Cit.3163.1.S1_at	3,67	up	CX641201	XP_478796.1	6E-74	putative adenosine-5 -phosphosulfate kinase [Oryza sativa (japonica cultivar-group)]
Cit.5718.1.S1_at	3,04	up	CX075009	AAT66764.1	7E-45	putative anaphase promoting complex protein [Solanum demissum]
Cit.14765.1.S1_at	3,15	up	CN183604	CAA74753.1	2E-88	putative arabinose kinase [Arabidopsis thaliana]
Cit.25753.1.S1_at	5,04	up	CF831703	NP_909181.1	2E-44	putative aspartic proteinase nepenthesin I [Oryza sativa (japonica cultivar-group)]
Cit.15840.1.S1_at	6,31	up	CV717225	NP_910416.1	1E-102	putative ATP-dependent proteinase LON2 [Oryza sativa (japonica cultivar-group)]
Cit.9875.1.S1_at	3,41	up	CX043939	XP_464513.1	2E-27	putative BRI1-KD interacting protein [Oryza sativa (japonica cultivar-group)]
Cit.39577.1.S1_at	3,64	up	DN795743	BAD36111.1	2E-15	putative BRI1-KD interacting protein 118 [Oryza sativa (japonica cultivar-group)]
Cit.10737.1.S1_s_at	3,18	up	CX643843	AAD43166.1	1E-55	Putative BURP domain containing protein [Arabidopsis thaliana]
Cit.39619.1.S1_s_at	3,85	up	DN799085	NP_915149.1	2E-57	putative calreticulin [Oryza sativa (japonica cultivar-group)]
Cit.16018.1.S1_at	4,04	up	CV710299	BAC43578.1	3E-57	putative CCHC-type zinc finger protein [Arabidopsis thaliana]
Cit.15008.1.S1_at	4,31	up	CX053346	XP_479002.1	7E-24	putative cyclin-dependent kinase CDC2C [Oryza sativa (japonica cultivar-group)]
Cit.1485.1.S1_at	3,36	up	CF832352	AAM64879.1	2E-58	putative cytochrome c oxidase subunit Vb [Arabidopsis thaliana]
Cit.5767.1.S1_at	3,40	up	CV719894	AAM61354.1	1E-105	putative cytochrome P450 [Arabidopsis thaliana]
Cit.2994.1.S1_s_at	7,37	up	CX543268	CAA71517.1	1E-83	putative cytochrome P450 [Glycine max]
Cit.5903.1.S1_at	3,14	up	CX077074	NP_913585.1	4E-29	putative DKFZP564O0463 protein [Oryza sativa (japonica cultivar-group)]
Cit.32821.1.S1_at	4,13	up	CX294900	XP_465943.1	1E-57	putative DNA polymerase epsilon catalytic subunit protein isoform b [Oryza sativa (japonica cultivar-group)]
Cit.7845.1.S1_at	3,75	up	CX676186	BAD31595.1	2E-13	putative fanconi anemia, complementation group D2 [Oryza sativa (japonica cultivar-group)]
Cit.16844.1.S1_at	3,74	up	CX637510	AAK59670.2	4E-47	putative FtsH protease [Arabidopsis thaliana]
Cit.6257.1.S1_at	3,62	up	CX045914	BAD44667.1	4E-83	putative glycerate dehydrogenase [Arabidopsis thaliana]
Cit.15378.1.S1_at	4,58	up	CX294788	AAM63425.1	8E-91	putative glycosylation enzyme [Arabidopsis thaliana]
Cit.13998.1.S1_s_at	5,22	up	CX670641	BAC22314.1	1E-133	putative GMP synthetase [Oryza sativa (japonica cultivar-group)]
Cit.16519.1.S1_at	3,07	up	CX669582	AAC63846.1	2E-62	putative GTP-binding protein [Arabidopsis thaliana]
Cit.32245.1.S1_at	3,35	up	CX289661	AAM20391.1	3E-15	putative homeobox protein [Arabidopsis thaliana]
Cit.15386.1.S1_at	3,39	up	CV710376	XP_478449.1	1E-57	putative ionotropic glutamate receptor homolog GLR4 [Oryza sativa (japonica cultivar-group)]

Cit.30068.1.S1_at	3,28	up	CF504921	BAC43244.1	2E-31	putative kinase binding protein [Arabidopsis thaliana]
Cit.4511.1.S1_s_at	3,30	up	CX664995	AAM51291.1	4E-04	putative membrane import protein [Arabidopsis thaliana]
Cit.10908.1.S1_s_at	3,67	up	CD575566	AAN13106.1	1E-105	putative mitochondrial dicarboxylate carrier protein [Arabidopsis thaliana]
Cit.10907.1.S1_at	4,20	up	CK937084	AAN13106.1	1E-111	putative mitochondrial dicarboxylate carrier protein [Arabidopsis thaliana]
Cit.5285.1.S1_s_at	3,89	up	CX053999	NP_912438.1	5E-60	Putative mitochondrial inner membrane protein [Oryza sativa (japonica cultivar-group)]
Cit.6968.1.S1_at	3,74	up	CV712980	AAT39295.1	1E-98	putative origin recognition complex subunit 6 (ORC6) containing protein [Solanum demissum]
Cit.30729.1.S1_s_at	3,78	up	CX077698	AAF23225.1	1E-120	putative pectinacetylesterase [Arabidopsis thaliana]
Cit.10327.1.S1_s_at	3,05	up	CD574467	AAN12942.1	3E-74	putative peroxiredoxin [Arabidopsis thaliana]
Cit.12524.1.S1_s_at	3,20	up	CF836121	BAC42870.1	6E-81	putative phosphatidylinositol/ phosphatidylcholine transfer protein [Arabidopsis thaliana]
Cit.15079.1.S1_at	3,42	up	CV715181	AAC69277.2	5E-57	putative phospholipase A2 [Dianthus caryophyllus]
Cit.29672.1.S1_s_at	3,37	up	CX297211	AAM20329.1	1E-81	putative plastid protein [Arabidopsis thaliana]
Cit.7790.1.S1_at	3,60	up	CV708188	CAB96664.1	7E-34	putative protein [Arabidopsis thaliana]
Cit.25392.1.S1_s_at	3,15	up	DT214430	AAN17411.1	6E-50	putative protein [Arabidopsis thaliana]
Cit.16802.1.S1_at	3,01	up	CV713668	CAB79137.1	7E-21	putative protein [Arabidopsis thaliana]
Cit.16277.1.S1_at	4,03	up	CV713760	CAB68186.1	7E-66	putative protein [Arabidopsis thaliana]
Cit.12077.1.S1_at	4,26	up	CX673716	CAB66408.1	1E-102	putative protein [Arabidopsis thaliana]
Cit.16433.1.S1_at	4,37	up	CX077438	CAB77595.1	1E-20	putative protein [Arabidopsis thaliana]
Cit.34184.1.S1_s_at	3,25	up	CX305043	CAB86629.1	1E-26	putative protein [Arabidopsis thaliana]
Cit.28706.1.S1_at	5,19	up	CV711966	CAB86633.1	1E-29	putative protein [Arabidopsis thaliana]
Cit.16250.1.S1_at	3,40	up	CX045320	CAB75464.1	5E-41	putative protein [Arabidopsis thaliana]
Cit.16661.1.S1_at	4,65	up	CX674414	CAB88257.1	6E-46	putative protein [Arabidopsis thaliana]
Cit.7094.1.S1_at	4,77	up	DN618365	CAB63022.1	1E-17	putative protein [Arabidopsis thaliana]
Cit.9857.1.S1_at	3,17	up	CX544425	AAC26685.1	1E-102	putative pyruvate dehydrogenase E1 beta subunit [Arabidopsis thaliana]
Cit.15552.1.S1_at	3,76	up	CN182046	AAP03381.1	3E-76	putative pyruvate kinase [Oryza sativa (japonica cultivar-group)]
Cit.13717.1.S1_at	3,07	up	CX636366	AAK92807.1	1E-124	putative receptor protein kinase [Arabidopsis thaliana]
Cit.30112.1.S1_at	4,04	up	CF506825	AAK59558.1	7E-15	putative receptor-protein kinase [Arabidopsis thaliana]
Cit.4221.1.S1_at	4,41	up	CX043598	AAM20379.1	1E-106	putative ripening protein [Arabidopsis thaliana]
Cit.30496.1.S1_s_at	4,10	up	DR403254	CAB85635.1	5E-62	putative ripening-related P-450 enzyme [Vitis vinifera]
Cit.14534.1.S1_at	4,04	up	CX635600	CAB85635.1	5E-67	putative ripening-related P-450 enzyme [Vitis vinifera]
Cit.2572.1.S1_at	3,17	up	CX672654	AAN15403.1	1E-118	putative RNA-binding protein [Arabidopsis thaliana]
Cit.12227.1.S1_at	5,43	up	CV714937	AAM61313.1	2E-43	putative RNA-binding protein [Arabidopsis thaliana]
Cit.12226.1.S1_s_at	3,91	up	DN622183	AAM61313.1	4E-44	putative RNA-binding protein [Arabidopsis thaliana]
Cit.1110.1.S1_at	3,58	up	CX668949	BAD28559.1	1E-106	putative Sec61 alpha form 2 [Oryza sativa (japonica cultivar-group)]
Cit.39268.1.S1_s_at	4,73	up	DN960037	CAB80000.1	1E-122	putative serine/threonine protein kinase [Arabidopsis thaliana]
Cit.13249.1.S1_at	3,62	up	CV706603	XP_475526.1	1E-90	putative SF16 protein [Oryza sativa (japonica cultivar-group)]
Cit.3641.1.S1_s_at	3,53	up	CX643316	AAL85143.1	2E-14	putative small zinc finger protein TIM9 [Arabidopsis thaliana]
Cit.22172.1.S1_at	3,28	up	CX665113	XP_477792.1	5E-06	putative SNF2 domain/helicase domain-containing protein [Oryza sativa (japonica cultivar-group)]
Cit.2714.1.S1_at	3,34	up	CX673310	AAT77033.1	1E-148	putative TCP-1/cpn60 chaperonin family protein [Oryza sativa (japonica cultivar-group)]
Cit.2715.1.S1_at	3,52	up	CX667385	AAT77033.1	4E-75	putative TCP-1/cpn60 chaperonin family protein [Oryza sativa (japonica cultivar-group)]
Cit.3118.1.S1_s_at	3,90	up	CX043363	NP_921813.1	1E-123	putative thiolase [Oryza sativa (japonica cultivar-group)]
Cit.21156.1.S1_s_at	3,41	up	DN799292	XP_464429.1	1E-18	putative thioredoxin peroxidase [Oryza sativa (japonica cultivar-group)]
Cit.38119.1.S1_at	4,19	up	CF505665	AAO17013.1	1E-33	Putative thymidine kinase [Oryza sativa (japonica cultivar-group)]
Cit.5467.1.S1_at	3,28	up	CX051811	XP_480159.1	1E-123	putative U3 snoRNP protein IMP4 [Oryza sativa (japonica cultivar-group)]
Cit.11057.1.S1_s_at	3,58	up	CV716755	AAT40548.1	3E-83	putative vicilin [Solanum demissum]
Cit.1194.1.S1_s_at	5,46	up	CF833252	AAM28295.1	7E-18	PVR3-like protein [Ananas comosus]
Cit.9020.1.S1_s_at	3,06	up	CX305834	AAP37971.1	5E-20	seed specific protein Bn15D18B [Brassica napus]
Cit.35639.1.S1_at	3,78	up	CK939959	NP_188488.1	6E-75	single-strand-binding family protein [Arabidopsis thaliana]
Cit.17250.1.S1_s_at	5,24	up	CX288012	AAD39991.1	4E-27	small zinc finger-like protein [Malus x domestica]
Cit.12232.1.S1_at	6,13	up	CX054002	T11577	1E-153	type IIIa membrane protein cp-wap13 - cowpea
Cit.30649.1.S1_s_at	3,02	up	CX044904	AAQ87022.1	1E-107	VDAC2.1 [Lotus corniculatus var. japonicus]
Cit.22556.1.S1_s_at	3,10	up	CF833755	AAQ87022.1	5E-25	VDAC2.1 [Lotus corniculatus var. japonicus]
Cit.15834.1.S1_at	3,23	up	CX071432	CAB80043.1	1E-134	WD-repeat protein-like protein [Arabidopsis thaliana]
Cit.13686.1.S1_s_at	4,78	up	CX073116	CAB80044.1	1E-131	WD-repeat protein-like protein [Arabidopsis thaliana]

Unclassified

Probe Set ID	FC	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.14398.1.S1_at	3,62	up	CX671384	AAR24770.1	1E-77	At3g54130 [Arabidopsis thaliana]
Cit.4940.1.S1_at	3,39	up	CV714121	AAV43784.1	8E-73	At3g60510 [Arabidopsis thaliana]
Cit.18266.1.S1_at	4,72	up	CX073857	BAD52879.1	5E-87	ATP synthase beta subunit/transcription termination factor rho-like [Oryza sativa (japonica cultivar-group)]
Cit.27955.1.S1_at	4,19	up	CX052139	XP_468126.1	5E-26	BRUSHY1-like [Oryza sativa (japonica cultivar-group)]
Cit.2474.1.S1_at	3,07	up	CV884924	BAA84071.1	3E-39	cytochrome P450 [Antirrhinum majus]
Cit.35116.1.S1_s_at	4,59	up	CK933584	AAD47832.1	2E-45	cytochrome P450 [Nicotiana tabacum]
Cit.1814.1.S1_s_at	4,20	up	CX663419	O22307	8E-83	Cytochrome P450 71D11 gb|AAB69644.1| putative cytochrome P450 [Lotus japonicus]

Cit.5920.1.S1_at	3,81	up	CX663922	T52256	3E-80	cytochrome P-450LXXIA1 [similarity] - avocado gb|AAA32913.1| cytochrome P-450LXXIA1 (cyp71A1)
Cit.10778.1.S1_at	3,12	up	CX665203	AAR06239.1	1E-162	dicarboxylate/tricarboxylate carrier [Citrus junos]
Cit.11285.1.S1_s_at	3,11	up	CX639897	AAM65173.1	2E-11	endosperm specific protein-like [Arabidopsis thaliana]
Cit.17875.1.S1_s_at	3,00	up	CK940100	CAC39620.1	3E-59	ferredoxin-thioredoxin-reductase catalytic subunit B [Solanum tuberosum]
Cit.11658.1.S1_at	3,47	up	CX052754	CAC39620.1	5E-60	ferredoxin-thioredoxin-reductase catalytic subunit B [Solanum tuberosum]
Cit.32657.1.S1_at	3,33	up	CX293106	AAR13240.1	1E-107	KUP-related potassium transporter [Lotus corniculatus var. japonicus]
Cit.7423.1.S1_at	3,09	up	CX546504	CAA57831.1	3E-93	low affinity sulphate transporter [Stylosanthes hamata]
Cit.13362.1.S1_s_at	3,01	up	CF835622	NP_201035.1	8E-19	nucleotide-sensitive chloride conductance regulator (ICln) family protein [Arabidopsis thaliana]
Cit.1349.1.S1_s_at	3,01	up	CX071194	NP_915828.1	4E-80	P0003D09.18 [Oryza sativa (japonica cultivar-group)]
Cit.7810.1.S1_at	4,17	up	CX664696	CAC84774.1	5E-59	P70 protein [Nicotiana tabacum]
Cit.3708.1.S1_s_at	4,43	up	CX053295	BAD46607.1	4E-82	peptidylprolyl isomerase [Oryza sativa (japonica cultivar-group)]
Cit.11018.1.S1_s_at	3,76	up	CX076297	AAM65904.1	2E-93	peptidylprolyl isomerase-like protein [Arabidopsis thaliana]
Cit.3170.1.S1_at	3,19	up	CV707383	AAC62851.1	1E-83	photolyase/blue-light receptor (PHR2) [Arabidopsis thaliana]
Cit.1925.1.S1_s_at	4,76	up	CK936471	NP_194482.1	4E-64	plastocyanin-like domain-containing protein [Arabidopsis thaliana]
Cit.4773.1.S1_at	3,56	up	CD574636	AAF66825.1	3E-75	poly(A)-binding protein [Nicotiana tabacum]
Cit.25528.1.S1_at	3,02	up	CX668649	CAD45375.1	1E-83	potyviral helper component protease-interacting protein 2 [Solanum tuberosum subsp. andigena]
Cit.13736.1.S1_at	3,39	up	CX043377	XP_507251.1	5E-50	PREDICTED OJ1117_F10.8 gene product [Oryza sativa (japonica cultivar-group)]
Cit.26400.1.S1_s_at	3,06	up	CN185695	T00580	1E-78	probable [acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39) T27E13.6 [similarity] [Arabidopsis thaliana]
Cit.11322.1.S1_s_at	4,29	up	CX675896	AAG24908.1	1E-140	proliferating cell nuclear antigen [Nicotiana benthamiana]
Cit.9724.1.S1_at	3,08	up	CX072288	NP_198681.1	4E-52	proteasome maturation factor UMP1 family protein [Arabidopsis thaliana]
Cit.3300.1.S1_at	3,05	up	CN182855	Q9XG77	4E-64	Proteasome subunit alpha type 6 (20S proteasome alpha subunit A) (20S proteasome subunit alpha-1)
Cit.5521.1.S1_s_at	3,85	up	CF835073	NP_188232.1	3E-68	protein disulfide isomerase family [Arabidopsis thaliana]
Cit.6028.1.S1_at	3,19	up	DT214707	NP_177149.2	7E-89	protein kinase family protein [Arabidopsis thaliana]
Cit.8116.1.S1_at	3,17	up	CX294483	CAA47812.1	7E-72	ptxA [Pisum sativum] pir||T06482 probable cell wall protein - garden pea
Cit.10441.1.S1_at	4,31	up	CX635457	AAC67586.1	0.0	pyrophosphate-dependent phosphofructokinase beta subunit [Citrus x paradisi]
Cit.13055.1.S1_at	3,42	up	CX046366	AAM22747.1	2E-87	pyruvate kinase-like [Deschampsia antarctica]
Cit.12979.1.S1_at	3,76	up	CX669533	AAO45013.1	5E-62	S-adenosyl-L-methionine:benzoic acid/salicylic acid carboxyl methyltransferase [Petunia x hybrida]
Cit.6468.1.S1_at	3,41	up	CV887051	AAR06292.1	1E-132	SAICAR synthetase [Nicotiana tabacum]
Cit.12525.1.S1_at	4,29	up	CX298658	NP_973831.1	5E-82	SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein [Arabidopsis thaliana]
Cit.12029.1.S1_s_at	3,44	up	CX075198	BAD46280.1	7E-90	SEC14 cytosolic factor-like [Oryza sativa (japonica cultivar-group)]
Cit.28260.1.S1_at	3,03	up	DN619372	XP_216815.2	8E-30	similar to HCV NS3-transactivated protein 1 [Rattus norvegicus]
Cit.14702.1.S1_at	3,02	up	CX046703	NP_896728.1	5E-38	Sodium/bile acid cotransporter family [Synechococcus sp. WH 8102]
Cit.23756.1.S1_s_at	3,60	up	CV706025	BAD32780.1	2E-40	somatic embryogenesis receptor kinase 1 [Citrus unshiu]
Cit.11696.1.S1_at	3,75	up	CX075909	BAD32780.1	1E-101	somatic embryogenesis receptor kinase 1 [Citrus unshiu]
Cit.21857.1.S1_at	3,38	up	CB304535	NP_182038.1	2E-27	SPX (SYG1/Pho81/XPR1) domain-containing protein [Arabidopsis thaliana]
Cit.18181.1.S1_at	3,94	up	CF653284	NP_566473.2	4E-27	subtilase family protein [Arabidopsis thaliana]
Cit.28753.1.S1_s_at	3,02	up	CD573881	NP_567972.1	7E-36	subtilase family protein [Arabidopsis thaliana]
Cit.10653.1.S1_s_at	3,24	up	CD574431	S52770	3E-71	subtilisin-like proteinase (EC 3.4.21.-), nodule-specific - Arabidopsis thaliana (fragment)
Cit.13671.1.S1_at	3,87	up	CN192429	BAA21657.1	6E-67	sulfate transporter [Arabidopsis thaliana]
Cit.12977.1.S1_at	3,76	up	CX635527	BAC43758.1	7E-16	tentative caffeine synthase 3 [Coffea arabica]
Cit.13632.1.S1_s_at	3,78	up	DN619772	AAF63825.1	2E-62	thioredoxin, putative [Arabidopsis thaliana]
Cit.200.1.S1_at	3,85	up	CX664463	AAD10219.1	8E-85	transketolase [Spinacia oleracea]
Cit.15896.1.S1_at	3,88	up	CX072503	BAB10389.1	1E-105	ubiquitin [Arabidopsis thaliana]
Cit.5287.1.S1_at	3,63	up	DN620887	BAB40967.1	1E-133	UDP-D-glucuronate carboxy-lyase [Pisum sativum]
Cit.13826.1.S1_at	4,20	up	CX045299	AAL33919.1	3E-75	UDP-glucose pyrophosphorylase [Amorpha fruticosa]
Cit.4035.1.S1_at	3,13	up	CV712964	AAM19355.1	1E-107	UOS1 [Pisum sativum]
Cit.5010.1.S1_at	3,47	up	CX072638	CAA72093.1	1E-110	uracil phosphoribosyltransferase [Nicotiana tabacum]

Commonly modulated by PthAs 2 and 4, fold change > 3

Auxin mobilization and signaling

Probe Set ID	PthA2	Reg	PthA4	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.31860.1.S1_s_at	8,26	up	7,19	up	AU300809	NR:S33621	5E-13	ADR11-2 protein - soybean (fragment)
Cit.35736.1.S1_s_at	3,19	up	5,23	up	CK937473	NR:Q9ZRA4	9E-76	Auxin-binding protein ABP19a precursor

Carbohydrate, Aminoacid and Nucleotide Metabolism

Probe Set ID	PthA2	Reg	PthA4	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.28009.1.S1_at	3,31	up	9,18	up	CX671808	NR:NP_173510.1	1E-55	phosphate transporter family protein [Arabidopsis thaliana]
Cit.28009.1.S1_s_at	3,14	up	8,71	up	CX671808	NR:NP_173510.1	1E-55	phosphate transporter family protein [Arabidopsis thaliana]

Cell division and morphogenesis

Probe Set ID	PthA2	Reg	PthA4	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.3665.1.S1_at	5,46	up	11,84	up	CF508354	NR:CAB45241.1	7E-34	GEG protein [Gerbera hybrid cultivar]
Cit.3665.1.S1_s_at	3,34	up	6,23	up	CF508354	NR:CAB45241.1	7E-34	GEG protein [Gerbera hybrid cultivar]
Cit.29007.1.S1_at	5,32	up	4,43	up	CX637545	NR:S71371	4E-35	gibberellin-regulated protein GASA5 precursor [Arabidopsis thaliana]
Cit.18235.1.S1_at	7,83	up	9,48	up	CK936113	NR:NP_187298.1	2E-92	GNS1/SUR4 membrane family protein [Arabidopsis thaliana]
Cit.4118.1.S1_s_at	3,18	up	7,52	up	CK932935	NR:CAE02924.1	2E-69	OSJNBb0108J11.17 [Oryza sativa (japonica cultivar-group)]
Cit.5300.1.S1_at	3,87	up	11,71	up	CV713508	NR:AAL34163.1	5E-70	putative dUTP pyrophosphatase [Arabidopsis thaliana]
Cit.12502.1.S1_s_at	3,61	up	4,39	up	CX050861	NR:AAT39315.1	2E-90	putative senescence-associated protein [Solanum demissum]
Cit.11057.1.S1_s_at	4,93	up	3,56	up	CV716755	NR:AAT40548.1	3E-83	putative vicilin [Solanum demissum]
Cit.12503.1.S1_at	4,46	up	4,84	up	DN135183	NR:NP_190146.1	3E-82	senescence-associated family protein [Arabidopsis thaliana]

Cell-wall synthesis and remodelling

Probe Set ID	PthA2	Reg	PthA4	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.6334.1.S1_at	4,46	up	6,32	up	CV715984	NR:BAB10434.1	5E-99	ABC transporter-like protein [Arabidopsis thaliana]
Cit.2392.1.S1_at	81,46	up	42,38	up	CF831790	NR:AAB65155.1	0.0	acidic cellulase [Citrus sinensis]
Cit.14913.1.S1_s_at	3,41	up	8,32	up	CX043703	NR:CAA77656.1	6E-73	acidic chitinase III [Nicotiana tabacum]
Cit.14522.1.S1_at	3,29	up	3,60	up	CX043829	NR:CAA10382.2	1E-82	alpha-D-xylosidase [Tropaeolum majus]
Cit.23967.1.S1_s_at	3,48	up	3,31	up	CV710106	NR:CAA10382.2	4E-60	alpha-D-xylosidase [Tropaeolum majus]
Cit.399.1.S1_s_at	3,12	up	3,81	up	CF837795	NR:AAR09170.1	2E-45	alpha-expansin 3 [Populus tremula x Populus tremuloides]
Cit.2701.1.S1_at	3,61	up	6,67	up	CV710968	NR:AAO92753.1	2E-56	arabinogalactan protein [Gossypium hirsutum]
Cit.2700.1.S1_s_at	4,40	up	7,80	up	CV709336	NR:AAO92753.1	7E-81	arabinogalactan protein [Gossypium hirsutum]
Cit.19350.1.S1_s_at	4,24	up	4,12	up	CK934803	NR:NP_196320.1	4E-42	aspartyl protease family protein [Arabidopsis thaliana]
Cit.20158.1.S1_x_at	5,89	up	7,61	up	CF834901	NR:NP_191008.1	7E-23	aspartyl protease family protein [Arabidopsis thaliana]
Cit.11898.1.S1_at	3,39	up	8,43	up	CD573639	NR:AAL57631.1	4E-69	At1g78060/F28K19_32 [Arabidopsis thaliana]
Cit.9706.1.S1_s_at	3,79	up	23,17	up	CV886686	NR:CAA03908.1	1E-145	beta-1,3-glucanase [Citrus sinensis]
Cit.4483.1.S1_s_at	8,94	up	14,46	up	CX077158	NR:BAB11424.1	5E-79	beta-xylosidase [Arabidopsis thaliana]
Cit.3554.1.S1_s_at	6,83	up	6,94	up	CX663293	NR:T07885	1E-124	cellulase (EC 3.2.1.4) - sweet orange
Cit.21654.1.S1_s_at	3,11	up	9,17	up	CX299958	NR:AAC35981.1	5E-27	chitinase CHI1 [Citrus sinensis]
Cit.10594.1.S1_at	3,28	up	9,77	up	DN624837	NR:AAC35981.1	1E-78	chitinase CHI1 [Citrus sinensis]
Cit.15242.1.S1_at	4,68	up	10,38	up	CX292066	NR:AAC35981.1	1E-101	chitinase CHI1 [Citrus sinensis]
Cit.7877.1.S1_at	4,16	up	5,19	up	CX667721	NR:S48032	5E-95	cim1 protein - soybean
Cit.11915.1.S1_s_at	6,92	up	7,47	up	CX071344	NR:AAC17605.1	6E-45	Contains similarity to proline-rich protein
Cit.2945.1.S1_s_at	4,49	up	5,74	up	CV887291	NR:CAC94006.1	1E-148	endo-beta-1,4-glucanase [Fragaria x ananassa]
Cit.35756.1.S1_at	26,74	up	10,54	up	CB250319	NR:AAC64184.1	2E-96	endopolygalacturonase [Prunus persica]
Cit.32767.1.S1_at	35,41	up	11,70	up	CX294670	NR:AAP21999.1	1E-58	endopolygalacturonase [Prunus persica]
Cit.1385.1.S1_s_at	3,03	up	5,42	up	CV887009	NR:T10737	7E-67	extensin-like cell wall protein - sea-island cotton
Cit.1386.1.S1_at	3,99	up	6,89	up	CX672178	NR:T10737	5E-75	extensin-like cell wall protein - sea-island cotton
Cit.30534.1.S1_s_at	3,79	up	5,18	up	CK934786	NR:S65063	4E-21	fiber protein E6 (clones SIE6-2A and SIE6-3B) - sea-island cotton
Cit.17888.1.S1_s_at	4,89	up	8,51	up	CF838857	NR:NP_177929.1	1E-106	glycosyl hydrolase family 3 protein [Arabidopsis thaliana]
Cit.3390.1.S1_at	13,91	up	12,52	up	CV709535	NR:NP_197666.1	1E-128	glycosyl transferase family 2 protein [Arabidopsis thaliana]
Cit.1077.1.S1_s_at	4,77	up	4,31	up	CF829440	NR:AAQ84042.1	1E-93	pectate lyase [Malus x domestica]
Cit.17853.1.S1_s_at	7,42	up	5,50	up	CK933446	NR:NP_563715.1	1E-145	pectate lyase family protein [Arabidopsis thaliana]
Cit.26012.1.S1_at	9,44	up	5,84	up	CF833607	NR:AAF35897.1	8E-56	pectin methylesterase isoform alpha [Vigna radiata]
Cit.25554.1.S1_at	12,83	up	16,24	up	CX669376	NR:AAK50769.1	1E-63	polygalacturonase [Pisum sativum]
Cit.35754.1.S1_at	23,28	up	10,44	up	CB250305	NR:NP_191544.1	2E-09	polygalacturonase, putative [Arabidopsis thaliana]
Cit.4516.1.S1_s_at	4,59	up	7,36	up	CV886618	NR:AAP33475.1	1E-116	polygalacturonase-like protein [Fragaria x ananassa]
Cit.10437.1.S1_s_at	8,86	up	33,82	up	CX665316	NR:AAP33475.1	1E-144	polygalacturonase-like protein [Fragaria x ananassa]
Cit.20071.1.S1_s_at	6,58	up	20,93	up	CX666732	NR:AAP33475.1	4E-90	polygalacturonase-like protein [Fragaria x ananassa]
Cit.21559.1.S1_s_at	10,21	up	34,76	up	CX051882	NR:AAP33475.1	4E-33	polygalacturonase-like protein [Fragaria x ananassa]
Cit.311.1.S1_s_at	5,47	up	3,64	up	CN182741	NR:BAB16431.1	8E-18	P-rich protein NtEIG-C29 [Nicotiana tabacum]
Cit.14266.1.S1_at	3,04	up	4,17	up	CX666282	NR:CAB88664.1	1E-164	putative glucosyltransferase [Cicer arietinum]
Cit.13410.1.S1_s_at	4,38	up	9,14	up	CX669534	NR:AAP04044.1	1E-103	putative pectin methylesterase [Arabidopsis thaliana]
Cit.12490.1.S1_s_at	6,13	up	22,09	up	CX044619	NR:AAM20001.1	1E-134	putative polygalacturonase PG1 [Arabidopsis thaliana]
Cit.11685.1.S1_s_at	8,00	up	11,28	up	CX663825	NR:AAM65121.1	5E-74	putative proline-rich cell wall protein [Arabidopsis thaliana]

Cit.2949.1.S1_s_at	10,91	up	4,49	up	CN182557	NR:T10523	1E-153	xyloglucan endo-1,4-beta-D-glucanase (EC 3.2.1.-) 1 - common nasturtium
Disease resistance, defence and stress response								
Probe Set ID	**PthA2**	**Reg**	**PthA4**	**Reg**	**Rep Public ID**	**Protein ID**	**e-value**	**Target Description**
Cit.10661.1.S1_at	3,13	up	4,51	up	CB291294	NR:BAB82419.1	0.0	acid invertase [Citrus unshiu]
Cit.4457.1.S1_s_at	3,28	up	6,19	up	CD574780	NR:AAP03023.1	3E-82	acyl-activating enzyme 12 [Arabidopsis thaliana]
Cit.8908.1.S1_s_at	3,36	up	5,19	up	CX674698	NR:AAC39480.1	3E-92	aquaporin [Vernicia fordii]
Cit.4661.1.S1_s_at	4,72	up	8,23	up	CV713066	NR:AAT68601.1	1E-118	benzoyl coenzyme A: benzyl alcohol benzoyl transferase [Petunia x hybrida]
Cit.14757.1.S1_at	3,30	up	8,59	up	CK702467	NR:NP_566082.1	6E-37	calcium-binding protein, putative [Arabidopsis thaliana]
Cit.11691.1.S1_at	3,50	up	6,13	up	CF832883	NR:CAB42906.1	3E-48	calmodulin-like protein [Arabidopsis thaliana]
Cit.2113.1.S1_at	3,31	up	9,24	up	CK934775	NR:NP_176113.1	2E-59	disease resistance-responsive protein-related / dirigent protein-related [Arabidopsis thaliana]
Cit.11230.1.S1_s_at	3,37	up	6,46	up	CK936665	NR:BAB16426.1	4E-78	elicitor inducible gene product EIG-I24 [Nicotiana tabacum]
Cit.5769.1.S1_at	13,57	up	35,44	up	CN189092	NR:AAK62346.1	3E-57	elicitor-inducible cytochrome P450 [Nicotiana tabacum]
Cit.9584.1.S1_x_at	3,13	up	6,23	up	DR403286	NR:AAG30140.1	9E-83	glutathione S-transferase [Arabidopsis thaliana]
Cit.9301.1.S1_s_at	3,31	up	12,26	up	CN190145	NR:BAD11070.1	1E-102	HSR203J like protein [Capsicum chinense]
Cit.5956.1.S1_at	3,31	up	10,38	up	CV885460	NR:AAM18791.1	8E-31	immuno-reactant natriuretic peptide-like protein [Erucastrum strigosum]
Cit.10864.1.S1_s_at	3,87	up	4,55	up	CV706080	NR:CAE76632.1	3E-89	leucine rich repeat protein [Cicer arietinum]
Cit.2848.1.S1_s_at	5,20	up	3,01	up	DN795261	NR:NP_188718.1	1E-97	leucine-rich repeat family protein [Arabidopsis thaliana]
Cit.2848.1.S1_at	6,56	up	3,66	up	DN795261	NR:NP_188718.1	1E-97	leucine-rich repeat family protein [Arabidopsis thaliana]
Cit.26982.1.S1_at	3,80	up	3,42	up	CV716390	NR:NP_189066.1	1E-92	leucine-rich repeat transmembrane protein kinase, putative [Arabidopsis thaliana]
Cit.21128.1.S1_at	3,26	up	11,41	up	DN799150	NR:BAB02603.1	4E-27	leucoanthocyanidin dioxygenase-like protein [Arabidopsis thaliana]
Cit.26307.1.S1_s_at	9,09	up	12,48	up	CV884507	NR:CAH03799.1	4E-26	lipid transfer protein [Citrus sinensis]
Cit.17919.1.S1_s_at	3,03	up	4,99	up	DN795283	NR:Q93ZH0	1E-106	LysM-domain GPI-anchored protein 1 precursor
Cit.29369.1.S1_x_at	3,72	up	8,33	up	DR405740	NR:Q39967	1E-18	Major latex allergen Hev b 5
Cit.16900.1.S1_x_at	4,37	up	11,96	up	CB610530	NR:Q39967	6E-21	Major latex allergen Hev b 5
Cit.40341.1.S1_x_at	4,44	up	11,00	up	DR405740	NR:Q39967	1E-20	Major latex allergen Hev b 5
Cit.376.1.S1_x_at	4,58	up	11,74	up	CX666561	NR:Q39967	5E-22	Major latex allergen Hev b 5
Cit.100.1.S1_x_at	4,70	up	12,34	up	CK934397	NR:Q39967	3E-21	Major latex allergen Hev b 5
Cit.721.1.S1_x_at	5,30	up	14,64	up	CB290748	NR:Q39967	5E-22	Major latex allergen Hev b 5
Cit.31377.1.S1_at	5,15	up	13,37	up	DN619712	NR:AAB81668.1	8E-81	NAM (no apical meristem)-like protein [Arabidopsis thaliana]
Cit.106.1.S1_s_at	3,67	up	4,34	up	CF835209	NR:AAM62745.1	1E-116	nucleoid DNA-binding-like protein [Arabidopsis thaliana]
Cit.32625.1.S1_s_at	3,78	up	3,22	up	CX292843	NR:CAA58733.1	4E-07	PAR-1a [Nicotiana tabacum] pir||S57419 PAR-1a protein - common tobacco
Cit.23259.1.S1_s_at	3,83	up	6,69	up	CX676279	NR:AAB95118.1	3E-88	pathogenesis-related group 5 protein [Brassica rapa]
Cit.1200.1.S1_at	5,69	up	13,56	up	CX292655	NR:AAM21199.1	7E-90	pathogenesis-related protein 5-1 [Helianthus annuus]
Cit.15404.1.S1_at	13,62	up	53,39	up	CF653559	NR:AAK30143.1	2E-58	pathogenesis-related protein PR-1 precursor [Capsicum annuum]
Cit.31825.1.S1_at	4,25	up	4,45	up	AU300664	NR:NP_194308.1	6E-30	pathogenesis-related protein, putative [Arabidopsis thaliana]
Cit.8282.1.S1_s_at	3,81	up	4,51	up	CX303177	NR:AAD11482.1	1E-106	peroxidase precursor [Glycine max]
Cit.10235.1.S1_at	4,06	up	3,56	up	CN182037	NR:S62698	2E-64	photoassimilate-responsive protein precursor (clone PAR-1a) - common tobacco
Cit.19727.1.S1_x_at	3,84	up	7,35	up	CK937930	NR:BAB40143.1	4E-31	plasma membrane intrinsic protein 2-2 [Pyrus communis
Cit.14939.1.S1_at	5,33	up	18,09	up	CV718780	NR:XP_476016.1	1E-102	putative hypersensitive-induced response protein [Oryza sativa (japonica cultivar-group)]
Cit.18517.1.S1_at	4,71	up	20,18	up	CX293755	NR:AAO27256.1	4E-39	putative NADH-dehydrogenase [Pisum sativum]
Cit.18517.1.S1_s_at	3,07	up	11,34	up	CX293755	NR:AAO27256.1	4E-39	putative NADH-dehydrogenase [Pisum sativum]
Cit.19313.1.S1_at	5,56	up	21,42	up	CX308038	NR:EAL71975.1	2E-04	putative protein serine/threonine kinase [Dictyostelium discoideum]
Cit.19313.1.S1_s_at	3,35	up	12,11	up	CX308038	NR:EAL71975.1	2E-04	putative protein serine/threonine kinase [Dictyostelium discoideum]
Cit.5228.1.S1_s_at	3,18	up	5,22	up	DN622543	NR:AAK59558.1	1E-31	putative receptor-protein kinase [Arabidopsis thaliana]
Cit.2027.1.S1_s_at	3,70	up	5,23	up	DN617689	NR:AAD02832.1	1E-55	raffinose synthase [Cucumis sativus]
Cit.1820.1.S1_at	3,94	up	3,40	up	DN797852	NR:BAA21921.1	9E-37	ZPT2-12 [Petunia x hybrida]
Ethylene synthesis and signalling								
Probe Set ID	**PthA2**	**Reg**	**PthA4**	**Reg**	**Rep Public ID**	**Protein ID**	**e-value**	**Target Description**
Cit.4491.1.S1_at	3,26	up	8,05	up	CX643923	NR:CAB60722.1	0.0	ACC synthase [Citrus sinensis]
Putative Transcriptional Factors								
Probe Set ID	**PthA2**	**Reg**	**PthA4**	**Reg**	**Rep Public ID**	**Protein ID**	**e-value**	**Target Description**
Cit.8822.1.S1_s_at	5,26	up	6,14	up	CF835408	NR:AAN13013.1	6E-90	putative chloroplast nucleoid DNA-binding protein [Arabidopsis thaliana]
Cit.8823.1.S1_s_at	4,97	up	9,17	up	CN182354	NR:AAN13013.1	1E-113	putative chloroplast nucleoid DNA-binding protein [Arabidopsis thaliana]
Down Regulated								
Probe Set ID	**PthA2**	**Reg**	**PthA4**	**Reg**	**Rep Public ID**	**Protein ID**	**e-value**	**Target Description**
Cit.9020.1.S1_s_at	3,15	down	3,06	up	CX305834	NR:AAP37971.1	5E-20	seed specific protein Bn15D18B [Brassica napus]
Cit.13553.1.S1_at	3,04	down	3,37	down	DN797932	NR:AAL85105.1	2E-56	putative heat shock protein [Arabidopsis thaliana]
Cit.18116.1.S1_at	4,26	down	3,65	down	CF509924	NR:NP_199696.2	4E-35	male sterility MS5 family [Arabidopsis thaliana]
Cit.29321.1.S1_x_at	3,81	down	4,53	down	CK934292	NR:AAG38518.1	3E-59	miraculin-like protein 2 [Citrus x paradisi]
Cit.11435.1.S1_s_at	5,07	down	8,83	down	DN619965	NR:NP_566623.1	9E-69	oxidoreductase, 2OG-Fe(II) oxygenase family protein [Arabidopsis thaliana]
Cit.30664.1.S1_at	4,24	down	5,72	down	CX307271	NR:CAE03090.2	6E-10	OSJNBa0017B10.5 [Oryza sativa (japonica cultivar-group)]
Cit.30664.1.S1_x_at	3,87	down	5,27	down	CX307271	NR:CAE03090.2	6E-10	OSJNBa0017B10.5 [Oryza sativa (japonica cultivar-group)]
Cit.15018.1.S1_at	3,25	down	6,59	down	CX300360	NR:AAM63015.1	4E-21	probable wound-induced protein [Arabidopsis thaliana]

Cit.32002.1.S1_at	*3,41*	*down*	*5,72*	*down*	*CX287685*	*NR:BAB01805.1*	*3E-50*	*unnamed protein product [Arabidopsis thaliana]*
Cit.12618.1.S1_at	*3,07*	*down*	*3,40*	*down*	*CN187375*	*NR:AAP37970.1*	*3E-77*	*seed specific protein Bn15D17A [Brassica napus]*
Cit.18045.1.S1_s_at	*3,89*	*down*	*6,43*	*down*	*CN185220*	*NR:AAM63508.1*	*6E-25*	*transcription factor TINY, putative [Arabidopsis thaliana]*
Unknown/Hypothetical								
Probe Set ID	***PthA2***	***Reg***	***PthA4***	***Reg***	***Rep Public ID***	***Protein ID***	***e-value***	***Target Description***
Cit.10820.1.S1_at	*3,35*	*up*	*8,96*	*up*	*CF420987*	*NR:AAN18146.1*	*5E-95*	*At2g38710/T6A23.9 [Arabidopsis thaliana]*
Cit.39558.1.S1_s_at	*3,11*	*up*	*8,40*	*up*	*DN795545*	*NR:AAN18146.1*	*3E-81*	*At2g38710/T6A23.9 [Arabidopsis thaliana]*
Cit.24517.1.S1_at	*4,16*	*up*	*6,39*	*up*	*CX546022*	*NR:AAU05534.1*	*3E-28*	*At4g12320 [Arabidopsis thaliana]*
Cit.29003.1.S1_s_at	*3,64*	*up*	*4,88*	*up*	*CX637270*	*NR:AAR24736.1*	*4E-43*	*At4g32870 [Arabidopsis thaliana]*
Cit.5609.1.S1_at	*7,17*	*up*	*3,26*	*up*	*CV884719*	*NR:AAS76239.1*	*3E-52*	*At5g19340 [Arabidopsis thaliana]*
Cit.25089.1.S1_at	*4,52*	*up*	*9,37*	*up*	*CX306331*	*NR:BAD06417.1*	*1E-51*	*cytochrome P450 [Asparagus officinalis]*
Cit.12932.1.S1_s_at	*4,57*	*up*	*4,75*	*up*	*CF653202*	*NR:NP_194433.1*	*2E-89*	*expressed protein [Arabidopsis thaliana]*
Cit.29791.1.S1_at	*7,22*	*up*	*4,55*	*up*	*CK933221*	*NR:T10174*	*1E-117*	*hypothetical protein - castor bean emb|CAB02653.1| unknown [Ricinus communis]*
Cit.23561.1.S1_x_at	*3,13*	*up*	*3,66*	*up*	*DN620570*	*NR:XP_326282.1*	*3E-06*	*hypothetical protein [Neurospora crassa]*
Cit.30668.1.S1_s_at	*3,81*	*up*	*6,95*	*up*	*DN795568*	*NR:NP_913447.1*	*4E-19*	*P0492F05.24 [Oryza sativa (japonica cultivar-group)]*
Cit.1194.1.S1_s_at	*4,10*	*up*	*5,46*	*up*	*CF833252*	*NR:AAM28295.1*	*7E-18*	*PVR3-like protein [Ananas comosus]*
Cit.12232.1.S1_at	*3,62*	*up*	*6,13*	*up*	*CX054002*	*NR:T11577*	*1E-153*	*type IIIa membrane protein cp-wap13 - cowpea*

Gus Vs PthC1, fold change ≥3

Probe Set ID	PthC1	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.22649.1.S1_s_at	3,30	up	CF837021	T07086	6E-51	acid phosphatase (EC 3.1.3.-) - soybean
Cit.4441.1.S1_s_at	3,12	up	CV710224	AAN18208.1	1E-99	At2g29670/T27A16.23 [Arabidopsis thaliana]
Cit.14811.1.S1_at	3,17	up	CX544652	AAG43551.1	3E-34	Avr9/Cf-9 rapidly elicited protein 146 [Nicotiana tabacum]
Cit.39526.1.S1_s_at	3,03	up	DN795359	T07821	7E-42	Caقم2exchanging protein - mung bean
Cit.8933.1.S1_s_at	3,10	up	CX673755	2119166A	8E-69	caffeic acid O-methyltransferase
Cit.1517.1.S1_s_at	3,67	up	CF418034	S14305	1E-127	chlorophyll a/b-binding protein (cab-11) - tomato
Cit.38715.1.S1_s_at	3,44	up	CK701355	AAR85968.1	6E-24	ERT10 [Nicotiana tabacum]
Cit.994.1.S1_s_at	3,16	up	CN187913	AAL90750.1	3E-44	glutaredoxin [Populus tremula x Populus tremuloides]
Cit.3390.1.S1_at	3,18	up	CV709535	NP_197666.1	1E-128	glycosyl transferase family 2 protein [Arabidopsis thaliana]
Cit.8686.1.S1_at	3,84	up	CV718920	S00838	2E-37	hemoglobin - Trema tomentosa prf||1402313A hemoglobin
Cit.71.1.S1_s_at	3,66	up	CX676086	BAA25393.1	1E-142	light harvesting chlorophyll a/b-binding protein [Nicotiana sylvestris]
Cit.72.1.S1_s_at	3,04	up	CF833881	AAG38519.1	1E-112	miraculin-like protein 3 [Citrus x paradisi]
Cit.9939.1.S1_s_at	3,59	up	CN183674	BAA21089.1	1E-137	NADPH-protochlorophyllide oxidoreductase [Cucumis sativus]
Cit.9110.1.S1_at	3,64	up	CF836107	P17340	4E-68	Plastocyanin, chloroplast precursor pir||S05303 plastocyanin precursor - tomato
Cit.25374.1.S1_s_at	4,32	up	DN625814	BAC43689.1	7E-59	putative mitochondrion-localized small heat shock protein [Arabidopsis thaliana]
Cit.1842.1.S1_at	3,25	up	CX300551	CAD29821.2	1E-57	putative photosystem I reaction centre subunit IV [Populus euramericana]
Cit.5237.1.S1_s_at	3,31	up	CX070296	CAB65284.1	1E-20	putative wound-induced protein [Medicago sativa subsp. x varia]
Cit.9827.1.S1_s_at	3,22	up	CN181604	BAA89230.1	4E-73	wts2L [Citrullus lanatus]
Cit.13271.1.S1_at	3,33	down	CX671361	AAV28174.1	2E-73	aldo/keto reductase [Fragaria x ananassa]
Cit.13250.1.S1_s_at	3,82	down	DN625183	AAD49420.1	1E-144	amine oxidase [Canavalia lineata]
Cit.34096.1.S1_s_at	3,04	down	CX304446	BAA07663.1	9E-35	cationic peroxidase isozyme 38K precursor [Nicotiana tabacum]
Cit.8346.1.S1_s_at	3,01	down	CB290303	AAN78125.1	1E-133	dehydrin [Citrus x paradisi]
Cit.5769.1.S1_at	3,55	down	CN189092	AAK62346.1	3E-57	elicitor-inducible cytochrome P450 [Nicotiana tabacum]
Cit.21009.1.S1_s_at	3,24	down	DN795505	AAL68830.1	8E-20	Enod8.3 [Medicago truncatula]
Cit.19728.1.S1_s_at	3,01	down	CK937931	NP_194842.1	4E-37	expressed protein [Arabidopsis thaliana]
Cit.1668.1.S1_s_at	3,16	down	CN189603	AAF73132.1	1E-130	homogentisate 1,2-dioxygenase [Lycopersicon esculentum]
Cit.12543.1.S1_at	3,43	down	CX637083	AAD55473.1	4E-69	Hypothetical protein [Arabidopsis thaliana]
Cit.4216.1.S1_s_at	3,28	down	CX672299	CAA07575.1	3E-81	monooxygenase [Arabidopsis thaliana]
Cit.803.1.S1_s_at	3,17	down	CF836730	AAM20167.1	1E-144	putative beta-amylase [Arabidopsis thaliana]
Cit.24487.1.S1_s_at	3,04	down	CX545678	AAL33815.1	7E-48	putative serine-type carboxypeptidase II [Arabidopsis thaliana]
Cit.9020.1.S1_s_at	3,08	down	CX305834	AAP37971.1	5E-20	seed specific protein Bn15D18B [Brassica napus]

Gus Vs PthC2, fold change >3

Probe Set ID	PthC2	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.29330.1.S1_s_at	11,23	up	CK936768	NP_193130.1	1E-103	adenosylhomocysteinase / S-adenosyl-L-homocysteine hydrolase / AdoHcyase (SAHH) [Arabidopsis thaliana]
Cit.19350.1.S1_s_at	4,05	up	CK934803	NP_196320.1	4E-42	aspartyl protease family protein [Arabidopsis thaliana]
Cit.12699.1.S1_s_at	3,06	up	CX666741	AAP13435.1	9E-16	At1g20030 [Arabidopsis thaliana]
Cit.29632.1.S1_at	3,07	up	CN181652	AAL90949.1	1E-94	AT5g41970/MJC20_7 [Arabidopsis thaliana]
Cit.20985.1.S1_s_at	4,66	up	DN795113	AAM64219.1	7E-06	cadmium induced protein CdI19 [Arabidopsis thaliana]
Cit.19806.1.S1_s_at	3,58	up	CK936888	AAF66066.2	4E-35	cinnamate 4-hydroxylase CYP73 [Citrus sinensis]
Cit.5891.1.S1_at	4,05	up	CK939541	O80337	5E-62	Ethylene responsive element binding factor 1 (AtERF1) (EREBP-2 protein)
Cit.109.1.S1_x_at	3,13	up	CK935762	BAA92155.1	4E-73	glycine-rich protein [Citrus unshiu]
Cit.7989.1.S1_s_at	3,13	up	CN186621	AAB42159.1	1E-136	Hsc70 pir||JC4786 dnaK-type molecular chaperone hsc70-3 - tomato
Cit.12700.1.S1_x_at	3,10	up	CB290379	NP_173432.2	6E-61	pathogenesis-related thaumatin family protein [Arabidopsis thaliana]
Cit.39990.1.S1_s_at	3,25	up	CN191354	T05707	5E-62	phosphate transport protein G7, mitochondrial - soybean
Cit.40133.1.S1_s_at	4,19	up	CN184966	AAM91543.1	3E-29	phosphoserine aminotransferase [Arabidopsis thaliana]
Cit.29561.1.S1_at	5,49	up	AU186271	NP_190058.1	3E-14	prephenate dehydratase family protein [Arabidopsis thaliana]
Cit.29561.1.S1_x_at	4,92	up	AU186271	NP_190058.1	3E-14	prephenate dehydratase family protein [Arabidopsis thaliana]
Cit.25374.1.S1_s_at	3,19	up	DN625814	BAC43689.1	7E-59	putative mitochondrion-localized small heat shock protein [Arabidopsis thaliana]
Cit.3450.1.S1_at	3,09	up	DN617664	CAC42087.1	8E-84	putative NAC domain protein [Solanum tuberosum]
Cit.39093.1.S1_s_at	3,95	up	DN958552	BAD42345.1	2E-84	sorbitol transporter [Malus x domestica]
Cit.35157.1.S1_at	3,57	up	CK933255	AAR06921.1	2E-65	UDP-glycosyltransferase 89B2 [Stevia rebaudiana]
Cit.13263.1.S1_s_at	3,15	down	CX675603	AAM61494.1	4E-43	abscisic acid-induced-like protein [Arabidopsis thaliana]
Cit.9488.1.S1_at	3,11	down	CV705576	AAL55726.1	1E-168	alcohol dehydrogenase 2 [Vitis vinifera]
Cit.23845.1.S1_s_at	3,03	down	CV707405	AAO44040.1	8E-35	At4g31130 [Arabidopsis thaliana]
Cit.12728.1.S1_at	3,24	down	CX071636	NP_174368.1	1E-130	CTP synthase, putative / UTP--ammonia ligase, putative [Arabidopsis thaliana]
Cit.585.1.S1_s_at	3,72	down	CX641936	AAT84459.1	1E-45	cytochrome b5 isoform Cb5-B [Vernicia fordii]
Cit.14161.1.S1_at	3,32	down	CN184285	AAF79607.1	7E-58	F5M15.12 [Arabidopsis thaliana]
Cit.20225.1.S1_at	3,40	down	CX045593	AAF64423.1	3E-13	globulin-like protein [Cucumis melo]
Cit.6856.1.S1_s_at	3,26	down	BQ624816	CAB79380.1	7E-20	hypothetical protein [Arabidopsis thaliana]
Cit.19537.1.S1_s_at	3,07	down	CK939074	CAD56224.1	6E-08	hypothetical protein [Cicer arietinum]
Cit.38794.1.S1_at	3,16	down	CK702304	NP_188338.1	2E-39	invertase/pectin methylesterase inhibitor family protein [Arabidopsis thaliana]
Cit.610.1.S1_x_at	3,12	down	CB293615	AAB18227.1	1E-129	MipC [Mesembryanthemum crystallinum]
Cit.12214.1.S1_at	3,13	down	CX303148	AAM34774.1	3E-61	nam-like protein 11 [Petunia x hybrida]
Cit.18884.1.S1_at	3,15	down	CX301419	BAD18975.1	3E-16	phloroglucinol O-methyltransferase [Rosa chinensis var. spontanea]
Cit.23036.1.S1_s_at	10,12	down	CV714093	AAC61292.1	9E-30	GH3.1 Arabidopsis thaliana; putative auxin-regulated protein [Arabidopsis thaliana]
Cit.25302.1.S1_s_at	3,01	down	DN624503	XP_468438.1	2E-14	putative Bet1/Sft1-related SNARE [Oryza sativa (japonica cultivar-group)]
Cit.3588.1.S1_s_at	3,51	down	CN186755	AAM45052.1	3E-38	putative DNA binding protein ACBF [Arabidopsis thaliana]
Cit.11850.1.S1_at	3,20	down	CN182214	NP_920425.1	8E-95	putative hydroxyproline-rich glycoprotein [Oryza sativa (japonica cultivar-group)]
Cit.4403.1.S1_at	3,05	down	CN189035	AAM20155.1	2E-52	putative membrane transporter protein [Arabidopsis thaliana]
Cit.1194.1.S1_s_at	3,07	down	CF833252	AAM28295.1	7E-18	PVR3-like protein [Ananas comosus]
Cit.11883.1.S1_s_at	4,43	down	CN186861	NP_569030.1	1E-34	senescence-associated family protein [Arabidopsis thaliana]
Cit.25144.1.S1_at	3,95	down	CB304989	NP_179996.1	4E-45	short-chain dehydrogenase/reductase (SDR) family protein [Arabidopsis thaliana]
Cit.25144.1.S1_at	4,43	down	CB304989	NP_179996.1	4E-45	short-chain dehydrogenase/reductase (SDR) family protein [Arabidopsis thaliana]
Cit.31084.1.S1_at	3,69	down	CX304978	NP_188329.1	4E-81	tetratricopeptide repeat (TPR)-containing protein [Arabidopsis thaliana]

Commonly modulated by PthC1 and 2, fold change >3

Probe Set ID	PthC1	Reg	PthC2	Reg	Rep Public ID	Protein ID	e-value	Target Description
Cit.13437.1.S1_s_at	7,06	up	5,97	up	DN622894	AAD56042.1	0.0	ADP-glucose pyrophosphorylase large subunit [Citrus unshiu]
Cit.15251.1.S1_at	3,06	up	3,26	up	CX543471	AAU03365.1	2E-48	alpha/beta fold family protein [Lycopersicon esculentum]
Cit.10673.1.S1_at	4,12	up	4,06	up	CX672012	AAP23943.1	4E-75	CCR protein [x Citrofortunella mitis]
Cit.29329.1.S1_x_at	3,19	up	3,80	up	CK937181	B34013	1E-118	chlorophyll a/b-binding protein 5 - soybean
Cit.4810.1.S1_at	3,45	up	4,28	up	CX043799	T03927	7E-67	DNA binding protein S25-XP1 - common tobacco
Cit.2910.1.S1_at	3,66	up	3,83	up	CX673184	NP_197519.1	3E-47	expressed protein [Arabidopsis thaliana]
Cit.9625.1.S1_s_at	3,83	up	3,94	up	CX051466	XP_480437.1	4E-76	glucose-6-phosphate/phosphate translocator [Oryza sativa (japonica cultivar-group)]
Cit.14406.1.S1_at	3,51	up	3,04	up	DR405149	BAC42648.1	2E-24	GPI-anchored protein [Arabidopsis thaliana]
Cit.2738.1.S1_s_at	3,86	up	3,42	up	CN182109	CAD22154.1	4E-80	pherophorin-dz1 protein [Volvox carteri f. nagariensis]
Cit.12071.1.S1_s_at	3,24	up	3,01	up	CV713928	AAB80714.1	1E-162	phosphoenolpyruvate carboxylase 1 [Gossypium hirsutum]
Cit.1838.1.S1_s_at	6,03	up	3,75	up	CV715889	AAM63015.1	1E-24	probable wound-induced protein [Arabidopsis thaliana]
Cit.10585.1.S1_s_at	4,71	up	3,79	up	CX673652	AAM91745.1	6E-93	putative phosphoethanolamine N-methyltransferase [Arabidopsis thaliana]
Cit.465.1.S1_s_at	4,82	up	4,47	up	CB293890	CAB05370.1	1E-139	thi [Citrus sinensis]
Cit.22644.1.S1_at	4,36	up	4,04	up	CF836962	NP_567138.1	3E-77	tRNA isopentenyl transferase -related protein [Arabidopsis thaliana]
Cit.38046.1.S1_at	4,35	down	4,39	down	CF505025	AAD39596.1	3E-06	10A19I.11 [Oryza sativa (japonica cultivar-group)]
Cit.25102.1.S1_at	4,30	down	3,48	down	CX306727	CAA04670.1	7E-70	39 kDa EF-Hand containing protein [Solanum tuberosum]
Cit.1966.1.S1_s_at	4,14	down	4,06	down	CX665191	CAC14058.1	1E-126	acridone synthase [Ruta graveolens]
Cit.33964.1.S1_at	4,76	down	4,09	down	CX303075	Q39529	9E-43	Agglutinin II precursor (ClAII) (LecClAII)
Cit.3120.1.S1_s_at	4,43	down	4,47	down	CV719579	JC4320	1E-155	alcohol dehydrogenase (EC 1.1.1.1) - garden lettuce
Cit.1115.1.S1_s_at	3,46	down	3,24	down	CX543084	AAV28174.1	1E-86	aldo/keto reductase [Fragaria x ananassa]
Cit.13250.1.S1_at	3,70	down	3,02	down	DN625183	AAD49420.1	1E-144	amine oxidase [Canavalia lineata]
Cit.5512.1.S1_at	7,79	down	7,07	down	CX664109	AAR07518.1	5E-54	At1g12030 [Arabidopsis thaliana]
Cit.3033.1.S1_s_at	4,38	down	3,91	down	CV884349	AAP68226.1	1E-113	At1g67900 [Arabidopsis thaliana]
Cit.5933.1.S1_s_at	3,24	down	3,37	down	CX670988	AAL69455.1	1E-73	At2g41130/T3K9.10 [Arabidopsis thaliana]
Cit.10398.1.S1_at	3,91	down	3,89	down	CV712367	AAO42768.1	6E-68	At3g13050/MGH6_16 [Arabidopsis thaliana]
Cit.10425.1.S1_s_at	3,40	down	3,25	down	CD574397	AAP13385.1	2E-39	At3g56360 [Arabidopsis thaliana]
Cit.17300.1.S1_at	4,96	down	4,21	down	CK936132	AAK83611.1	2E-66	AT5g52420/K24M7_17 [Arabidopsis thaliana]
Cit.17300.1.S1_s_at	4,57	down	4,09	down	CK936132	AAK83611.1	2E-66	AT5g52420/K24M7_17 [Arabidopsis thaliana]
Cit.8963.1.S1_s_at	3,77	down	3,96	down	CV713157	CAC84706.1	5E-82	aux/IAA protein [Populus tremula x Populus tremuloides]
Cit.8966.1.S1_s_at	5,13	down	5,72	down	CV704184	CAC84706.1	2E-76	aux/IAA protein [Populus tremula x Populus tremuloides]
Cit.1599.1.S1_at	4,88	down	5,98	down	CF828380	AAG48763.1	2E-38	auxin-regulated protein IAA13 [Arabidopsis thaliana]
Cit.18018.1.S1_at	3,85	down	3,76	down	CK933306	NP_177688.1	2E-43	auxin-responsive protein, putative [Arabidopsis thaliana]
Cit.5636.1.S1_s_at	5,62	down	6,03	down	CX674585	AAQ74955.1	2E-62	Gbiaa-Re [Gossypium barbadense]
Cit.8972.1.S1_s_at	5,36	down	7,38	down	CK701644	CAD30274.1	1E-25	IAA16 protein [Gossypium hirsutum]
Cit.28480.1.S1_s_at	20,00	down	9,82	down	CV708106	AAS17751.1	2E-52	beta xylosidase [Fragaria x ananassa]
Cit.22468.1.S1_s_at	3,42	down	3,09	down	CB292431	AAD38148.1	4E-69	beta-amylase [Prunus armeniaca]
Cit.23972.1.S1_x_at	5,02	down	3,95	down	CV710307	AAD38148.1	2E-80	beta-amylase [Prunus armeniaca]
Cit.13265.1.S1_at	3,16	down	3,88	down	CN182471	NP_568246.1	4E-66	bZIP protein HY5 (HY5) [Arabidopsis thaliana]
Cit.6244.1.S1_at	6,66	down	6,20	down	CF829107	NP_568457.1	9E-44	bZIP transcription factor family protein [Arabidopsis thaliana]
Cit.17882.1.S1_s_at	4,96	down	5,00	down	CK936954	AAG24863.1	8E-48	CONSTANS-like protein [Ipomoea nil]
Cit.26113.1.S1_at	4,10	down	4,37	down	CF836727	NP_176957.1	4E-48	expressed protein [Arabidopsis thaliana]
Cit.4501.1.S1_s_at	3,79	down	5,07	down	CN188862	BAD08916.1	2E-32	calcium-binding EF-hand family protein-like [Oryza sativa (japonica cultivar-group)]
Cit.14757.1.S1_at	3,05	down	3,15	down	CK702467	NP_566082.1	6E-37	calcium-binding protein, putative [Arabidopsis thaliana]
Cit.1891.1.S1_at	3,07	down	4,34	down	CX297083	NP_567871.1	4E-94	ChaC-like family protein [Arabidopsis thaliana]
Cit.7080.1.S1_at	5,37	down	5,21	down	CN187357	NP_180917.1	2E-82	cinnamoyl-CoA reductase family [Arabidopsis thaliana]
Cit.8210.1.S1_s_at	4,05	down	3,31	down	CF831195	AAQ92310.1	2E-79	COR15 [Citrus clementina x Citrus reticulata]
Cit.10353.1.S1_at	3,87	down	4,07	down	CF834243	O48922	1E-121	Cytochrome P450 98A2
Cit.9998.1.S1_s_at	3,72	down	4,88	down	CX669483	BAA02724.1	4E-41	early nodulin [Glycine max]
Cit.5922.1.S1_at	3,22	down	3,46	down	CN186032	NP_973637.1	1E-119	endoplasmic reticulum oxidoreductin 1 (ERO1) family protein [Arabidopsis thaliana]
Cit.8403.1.S1_s_at	3,94	down	4,48	down	CN186375	NP_567775.1	1E-121	expressed protein [Arabidopsis thaliana]
Cit.19945.1.S1_at	3,55	down	3,04	down	CK935230	NP_568308.1	1E-55	expressed protein [Arabidopsis thaliana]
Cit.12932.1.S1_s_at	5,41	down	4,94	down	CF653202	NP_194433.1	2E-89	expressed protein [Arabidopsis thaliana]
Cit.19690.1.S1_x_at	7,49	down	9,29	down	CK938207	NP_190526.1	1E-13	expressed protein [Arabidopsis thaliana]
Cit.14560.1.S1_at	3,03	down	3,33	down	CF837668	NP_566972.1	9E-25	expressed protein [Arabidopsis thaliana]
Cit.14337.1.S1_at	3,81	down	5,24	down	CX297183	CAD47830.1	8E-43	hydroxycinnamoyl transferase [Nicotiana tabacum]
Cit.16401.1.S1_at	4,31	down	5,58	down	CX072993	AAC69953.1	3E-66	hypothetical protein [Arabidopsis thaliana]
Cit.16401.1.S1_s_at	5,05	down	5,71	down	CX072993	AAC69953.1	3E-66	hypothetical protein [Arabidopsis thaliana]
Cit.23470.1.S1_s_at	5,05	down	4,99	down	CX674613	CAH59411.1	1E-42	hypothetical protein [Plantago major]
Cit.17589.1.S1_x_at	3,04	down	3,13	down	DN799303	CAA86851.1	4E-48	Lea5 protein [Citrus sinensis]

Cit.12214.1.S1_s_at	5,85	down	5,76	down	CX303148	AAM34774.1	3E-61	nam-like protein 11 [Petunia x hybrida]
Cit.29416.1.S1_at	3,19	down	3,36	down	CX640994	NP_196822.1	1E-39	no apical meristem (NAM) family protein [Arabidopsis thaliana]
Cit.12252.1.S1_at	50,57	down	50,29	down	CF837443	AAD32141.1	1E-120	Nt-gh3 deduced protein [Nicotiana tabacum]
Cit.5201.1.S1_at	6,80	down	6,50	down	CF837666	AAD32141.1	1E-138	Nt-gh3 deduced protein [Nicotiana tabacum]
Cit.17407.1.S1_at	3,31	down	3,55	down	CK934325	AAD32145.1	4E-67	Nt-iaa4.5 deduced protein [Nicotiana tabacum]
Cit.2306.1.S1_at	9,17	down	3,51	down	CV714093	AAC61292.1	9E-30	putative auxin-regulated protein [Arabidopsis thaliana]
Cit.1579.1.S1_at	3,87	down	3,74	down	CF417649	AAU90342.1	4E-52	putative myb-like DNA-binding protein [Solanum demissum]
Cit.24522.1.S1_s_at	3,40	down	3,62	down	CX546067	AAU90342.1	7E-05	putative myb-like DNA-binding protein [Solanum demissum]
Cit.8195.1.S1_s_at	3,83	down	3,21	down	CN186645	CAE03090.2	2E-18	OSJNBa0017B10.5 [Oryza sativa (japonica cultivar-group)]
Cit.30571.1.S1_s_at	3,25	down	3,26	down	CX307150	CAE03655.2	2E-56	OSJNBa0060N03.20 [Oryza sativa (japonica cultivar-group)]
Cit.19177.1.S1_at	5,04	down	5,81	down	CX302923	NP_908927.1	5E-09	P0463A02.24 [Oryza sativa (japonica cultivar-group)]
Cit.17886.1.S1_s_at	3,26	down	3,23	down	CK939377	NP_915910.1	5E-05	P0468B07.6 [Oryza sativa (japonica cultivar-group)]
Cit.15404.1.S1_at	6,35	down	5,97	down	CF653559	AAK30143.1	2E-58	pathogenesis-related protein PR-1 precursor [Capsicum annuum]
Cit.858.1.S1_s_at	3,70	down	3,91	down	CV884382	AAK52084.1	1E-110	peroxidase [Nicotiana tabacum]
Cit.13378.1.S1_at	5,21	down	5,45	down	CX675059	NP_922759.1	1E-126	protein kinase REK [Oryza sativa (japonica cultivar-group)]
Cit.8231.1.S1_s_at	5,83	down	4,45	down	CF835866	NP_178298.2	1E-100	purple acid phosphatase, putative [Arabidopsis thaliana]
Cit.1718.1.S1_s_at	3,61	down	5,47	down	CV715281	AAL33783.1	9E-87	putative 1-aminocyclopropane-1-carboxylate oxidase [Arabidopsis thaliana]
Cit.31136.1.S1_at	3,42	down	3,48	down	CX299481	BAC42063.1	1E-114	putative cysteine proteinase [Arabidopsis thaliana]
Cit.6618.1.S1_at	3,16	down	3,17	down	CX665765	CAB79616.1	1E-07	putative DNA-binding protein [Arabidopsis thaliana]
Cit.165.1.S1_s_at	4,10	down	4,16	down	CB291284	AAO33591.1	1E-55	putative early light induced protein [Arachis hypogaea]
Cit.19769.1.S1_x_at	5,44	down	8,39	down	CK937268	AAO33591.1	4E-58	putative early light induced protein [Arachis hypogaea]
Cit.12930.1.S1_at	3,35	down	3,84	down	CV713823	CAB79558.1	7E-91	putative protein [Arabidopsis thaliana]
Cit.7295.1.S1_at	4,25	down	3,42	down	CX667737	CAB82975.1	2E-22	putative protein [Arabidopsis thaliana]
Cit.29843.1.S1_s_at	5,15	down	5,71	down	CX666948	BAC42147.1	2E-77	putative SCARECROW gene regulator [Arabidopsis thaliana]
Cit.22629.1.S1_s_at	4,66	down	4,68	down	CF836448	AAC69450.1	1E-72	putative serine/threonine protein kinase [Nicotiana tabacum]
Cit.5495.1.S1_at	3,73	down	3,68	down	DN797732	NP_199517.1	5E-22	senescence-associated protein-related [Arabidopsis thaliana]
Cit.14551.1.S1_s_at	4,05	down	4,07	down	CX674742	XP_397192.1	6E-04	similar to ENSANGP00000010721 [Apis mellifera]
Cit.21940.1.S1_s_at	3,17	down	3,56	down	CB305082	NP_565876.2	2E-59	SOUL heme-binding family protein [Arabidopsis thaliana]
Cit.6737.1.S1_at	6,02	down	3,57	down	CN185151	NP_568377.1	3E-59	sulfate transporter, putative [Arabidopsis thaliana]
Cit.31451.1.S1_s_at	3,84	down	3,76	down	BQ623844	AAD33596.1	8E-44	thioredoxin h [Hevea brasiliensis]
Cit.5818.1.S1_at	4,17	down	4,17	down	CX076979	DAA02290.1	4E-13	TPA: DVL19 [Arabidopsis thaliana]
Cit.20147.1.S1_at	3,33	down	3,75	down	CF833423	NP_199704.1	2E-10	transferase family protein [Arabidopsis thaliana]

– ANEXO II –

Participação do aluno como co-autor de um artigo científico publicado na revista *Proteins: Structure, Function, and Bioinformatics* (Murakami *et al.*, 2010). O trabalho versa sobre a investigação das características estruturais do domínio de repetição de PthA2 (*repeat domain of PthA2* ou RD2) por espalhamento de raios-X a baixo ângulo (*small-angle X-ray scattering* ou SAXS), acompanhado de demais estudos espectroscópicos. Além disso, o presente trabalho apresenta os resultados obtidos a partir do estudo de um peptídeo contendo 1,5 repetição do domínio interno de PthA2 por RMN.

ARTIGO CIENTÍFICO:

The repeat domain of the type III effector protein PthA shows a TPR-like structure and undergoes conformational changes upon DNA interaction

Mário Tyago Murakami, Mauricio Luis Sforça, Jorge Luiz Neves, Joice Helena Paiva, Mariane Noronha Domingues, André Luiz Araujo Pereira, Ana Carolina de Mattos Zeri, and Celso Eduardo Benedetti*

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PROTEINS
STRUCTURE ■ FUNCTION ■ BIOINFORMATICS

The repeat domain of the type III effector protein PthA shows a TPR-like structure and undergoes conformational changes upon DNA interaction

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ABSTRACT

Many plant pathogenic bacteria rely on effector proteins to suppress defense and manipulate host cell mechanisms to cause disease. The effector protein PthA modulates the host transcriptome to promote citrus canker. PthA possesses unusual protein architecture with an internal region encompassing variable numbers of near-identical tandem repeats of 34 amino acids termed the repeat domain. This domain mediates protein–protein and protein–DNA interactions, and two polymorphic residues in each repeat unit determine DNA specificity. To gain insights into how the repeat domain promotes protein–protein and protein–DNA contacts, we have solved the structure of a peptide corresponding to 1.5 units of the PthA repeat domain by nuclear magnetic resonance (NMR) and carried out small-angle X-ray scattering (SAXS) and spectroscopic studies on the entire 15.5-repeat domain of PthA2 (RD2). Consistent with secondary structure predictions and circular dichroism data, the NMR structure of the 1.5-repeat peptide reveals three α-helices connected by two turns that fold into a tetratricopeptide repeat (TPR)-like domain. The NMR structure corroborates the theoretical TPR superhelix predicted for RD2, which is also in agreement with the elongated shape of RD2 determined by SAXS. Furthermore, RD2 undergoes conformational changes in a pH-dependent manner and upon DNA interaction, and shows sequence similarities to pentatricopeptide repeat (PPR), a nucleic acid-binding motif structurally related to TPR. The results point to a model in which the RD2 structure changes its compactness as it embraces the DNA with the polymorphic diresidues facing the interior of the superhelix oriented toward the nucleotide bases.

Proteins 2010; 78:3386–3395.

Key words: TAL effectors; tetratricopeptide repeat; pentatricopeptide repeat (PPR); *Xanthomonas citri*.

INTRODUCTION

Xanthomonas axonopodis pv. *citri* (Xac), the causal agent of citrus canker, induces the formation of raised pustules on the surface of the host plant. The pustules typically develop into larger corky and water-soaked lesions, which break the epidermis favoring bacterial dissemination.[1,2] Although it is known that canker lesions result from the intense division and expansion of the host cells at the site of infection, how exactly Xac induces cell division and growth is not yet clear. We have shown that Xac modulates the synthesis of cell growth regulators during the onset of infection[3] and that the common action of auxin and gibberellin is required for initial canker development.[4] Interestingly, these hormones alone or in combination had no apparent effect on citrus cell growth or division, suggesting that another factor is required for canker formation.[4] This additional factor is thought to be the Xac effector protein PthA, which is sufficient to promote cell hypertrophy when transiently expressed in citrus leaves.[5] Accordingly, expression of PthA proteins in citrus cells provokes transcriptional changes that overlap with those triggered by Xac infection associated with auxin and gibberellin action (Pereira and Benedetti, unpublished data).

PthA proteins differ from each other primarily by the number of near-identical repeats of 33–34 amino acids that are tandemly located in the central region of the protein. This domain confers host selectivity and is critical to determine pathogenicity.[6,7] PthA proteins are 95–97% identical to AvrBs3, the best known *Xanthomonas* type III effector protein. AvrBs3 is targeted to the nucleus of host cells where it modu-

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lates transcription.[8] Notably, AvrBs3 recognizes different plant promoters and activates transcription of particular genes in both susceptible and resistant plants.[9,10] The interaction of AvrBs3 with its target DNA is mediated by its repeat domain,[9–12] which is also essential for AvrBs3 dimerization before its nuclear import.[13] Likewise, we found that the repeat domain of PthA proteins is critical for protein–protein interactions.[14] Therefore, it is becoming clear that the repeat domain of such effectors has a dual character as a protein scaffold that allows protein–protein and protein–DNA contacts with host targets.

An intriguing aspect of the repeat domain of such effectors is the variability found in every repeat unit at certain amino acid positions. In the case of the PthA variants, the polymorphism occurs preferentially at Positions 4 (D, E, Q, or A), 12 (N or H), 13 (I, D, G, or S), and 24 (R or A) of the repeat units.[14] Since most members of the AvrBs3/PthA protein family share high-sequence identity, it has been postulated that, besides the variation in the number of repeat units, the polymorphism within the repeat units would play a critical role in conferring the specificity required for the interactions with particular protein or DNA targets. Indeed, recent studies have demonstrated that the pair of residues at Positions 12–13 determines the DNA sequence specificity[15,16]; however, the structural basis for these interactions is presently unknown. Thus, structural information on the repeat region becomes highly relevant for understanding the molecular function of this unique protein domain. Although numerous homologues of AvrBs3/PthA proteins have been identified, no three-dimensional (3D) structure is yet available for any transcription activator-like (TAL) effector. Despite considerable efforts, we have not yet been able to crystallize any of the PthA variants or their repeat domains. Thus, we used a combination of circular dichroism (CD), dynamic light scattering (DLS), nuclear magnetic resonance (NMR), small-angle X-ray scattering (SAXS) and molecular modeling approaches to gain insights into the 3D structure of the PthA repeat domain. In this work, we present the first 3D structure of a peptide corresponding to 1.5-repeat units of PthA2 (PDB code: 2KQ5) and a low-resolution envelope for the entire repeat domain of PthA2 (RD2) that is in agreement with its theoretical tetratricopeptide repeat (TPR)-like structure. Spectroscopic studies also revealed that RD2 undergoes conformational changes in a pH-dependent manner and in the presence of DNA. Furthermore, we show that the repeat units of RD2 are similar to pentatricopeptide repeat (PPR), a 35 amino acid motif that is structurally related to TPR and have nucleic acid binding activity.[17,18]

MATERIALS AND METHODS

Protein expression and purification

The 15.5-RD2 was amplified from *X. citri* plasmids and cloned into the *Bam*HI/*Sac*I sites of pET28a (Novagen). The construct was sequenced and used to transform *Escherichia coli* strain BL21 (DE3). Protein expression was induced at OD_{600} = 0.6 with 0.4 m*M* IPTG at 25°C. The cells were harvested after 3 h, centrifuged, and suspended in binding buffer (20 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl, 15 m*M* imidazole, 5% (v/v) glycerol, 1 m*M* PMSF, 0.5% Nonidet P-40) and incubated on ice with lysozyme (1 mg mL^{-1}) and RNAse (10 μg mL^{-1}) for 30 min. Bacterial cells were disrupted by sonication and the soluble fraction was incubated with DNAse I (0.5 μg mL^{-1}) and $MgCl_2$ (2 m*M*). The suspension containing the 6xHis-RD2 was loaded on a 5-mL HiTrap chelating HP (GE Healthcare) column pre-equilibrated with binding buffer. The column was washed with 20 column volumes of the same buffer and the protein was eluted with imidazole gradient. After purification, dithiothreitol (DTT) was added to a final concentration of 1 m*M* and fractions displaying the highest purify were concentrated and loaded on a G75 16/60 Superdex (GE Healthcare) column, pre-equilibrated with 20 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl, 5% (v/v) glycerol, and 1 m*M* DTT. RD2 was eluted as a single peak with a molecular mass corresponding to the monomer.

Dynamic light scattering

DLS experiments were carried out using a DynaPro 810 (Protein Solutions) apparatus equipped with a Peltier module for temperature control. The wavelength of the laser light and the output power were set to 830 nm and 30 mW, respectively. About 100 measurements were made at intervals of 20 s for each run. The DLS experiments were repeated several times with intervals of 30 min to check stability. Protein solutions of 1 mg mL^{-1} were prepared in 50 m*M* sodium acetate pH 5.0, 50 m*M* sodium phosphate pH 6.0 and 7.0, and 20 m*M* Tris–HCl pH 8.0. The complex of RD2:DNA was prepared in a molar ratio of 1.0:1.2, incubated for 12 h at 20°C, and centrifuged at 10,000*g* for 15 min at 4°C before analysis. Standard curves of bovine serum albumin were used for calibration and the experiments were conducted at 18°C. Hydrodynamic parameters were determined using the software DYNAMICS v.6.10.1.2.

CD measurements

CD spectroscopy experiments were conducted on a JASCO J-810 CD spectrophotometer equipped with a Peltier temperature control using 1-mm path quartz cuvettes. Spectra were acquired with a final protein concentration of 5.0–10 μ*M* in 20 m*M* Tris–HCl pH 8.0, containing 1.0 m*M* DTT and 100 m*M* NaCl. The RD2:DNA mixes were incubated for 3 h at 20°C and centrifuged (10,000*g*) for 15 min at 4°C before analyses. CD measurements were collected between 185 and 260 nm using a scanning rate of 50 nm min^{-1} with an average response time of 4 s.

Secondary structure variations were monitored as a function of changes in the initial CD spectrum upon addition of sodium dodecyl sulfate (SDS) for the 1.5-repeat unit. For all the spectra, an average of 10 scans was accumulated and the background spectrum of the buffer was subtracted.

NMR studies

NMR experiments were performed at 20°C using a Varian Inova 600 MHz spectrometer equipped with a cryogenic probe. Synthetic peptides (Proteimax, São Paulo, Brazil) corresponding to 1.0- or 1.5-repeat units of the repeat domain of PthA were dissolved in 20 m*M* phosphate buffer, pH 5.0, containing 70 m*M* SDS, 1.0 m*M* DTT, and 5% (v/v) D_2O, at a final concentration of ~1.0 m*M*. Peptide resonance peaks were assigned using standard methods including correlation spectroscopy,[19] total correlation spectroscopy (TOCSY),[20] and nuclear Overhauser enhancement spectroscopy (NOESY).[21] The TOCSY spectra were acquired using a DIPSI spin-lock sequence at a field strength of 10 kHz and a mixing time of 70 ms. NOESY spectra were recorded with mixing times of 150 and 300 ms. All 2D experiments were acquired in the phase-sensitive mode using the method of states.[22] Water suppression was achieved by low-power continuous wave irradiation during the relaxation delay or using the WATERGATE method.[23] Data were processed and analyzed using the NMRPipe/NMRVIEW software.[24] Before Fourier transformation, the time domain data were zero filled in both dimensions to yield a (4096 by 4096) data matrix. When necessary, a fifth-order polynomial baseline correction was applied after transformation and phasing. To obtain distance constraints, crosspeak volumes were estimated from the NOESY spectra.

The structure of the peptide corresponding to 1.5-repeat units of PthA was calculated in a semiautomated iterative manner with the program CYANA version 2.1,[25] using 100 starting conformers. CYANA 2.1 protocol was applied to calibrate and assign NOE crosspeaks. After the first few rounds of automatic calculations, the NOESY spectra were analyzed again to identify additional crosspeaks consistent with the structural model and to correct misidentified NOEs. The structures obtained were further refined by restrained minimization and molecular dynamic (MD) studies using the CNS software.[26] The 20 structures with the lowest target function were selected to represent the ensemble of peptide structures. The quality of the structures was analyzed with PROCHECK-NMR.[27] The NMR data were deposited in the biological magnetic resonance bank (BMRB) and protein data bank (PDB) under the entry codes 16589 and 2KQ5, respectively.

SAXS data collection and analysis

SAXS data were collected at the D11A-SAXS beamline at the Brazilian Synchrotron Light Laboratory (LNLS). The radiation wavelength was set to 1.488 Å and a charge-coupled device area detector (MARCCD 165 mm) was used to record the scattering patterns. The sample-to-detector distance was set to 1415.95 mm to give a scattering vector ranging from 0.10 to 3.5 nm^{-1}. The measurements were carried out with a sample concentration of 2 mg mL^{-1} at 18°C. Each protein sample was previously analyzed by DLS and only monodisperse solutions (polydispersity <20%) were used. Protein samples were centrifuged for 15 min at 10,000*g* to eliminate any existing aggregates immediately before each measurement. The scattering curves of the protein solutions and buffers were collected in frames of 300 s each to avoid radiation-induced protein damage. Each frame was carefully checked for possible bubbles or radiation-induced aggregation of the protein before calculating the average intensity and the associated experimental error. The experimental intensities were corrected for background, buffer contributions, detector inhomogeneities, and sample transmission.

The radius of gyration (R_g) was evaluated using Guinier approximation[28] as implemented in the program PRIMUS.[29] The indirect Fourier transform package GNOM[30] was used to evaluate the pair-distance distribution function $p(r)$. The low-resolution envelope of the RD2 protein was determined using *ab initio* modeling implemented in DAMMIN.[31] An averaged model was generated from several runs using the DAMAVER suite of programs.[32] The SAXS model and the NMR structures were superimposed with SUPCOMB.[33]

Homology molecular modeling of the RD2

The crystal structures of importin β-1 from *Saccharomyces cerevisiae* (2BPT)[34] and TIP120 from *Homo sapiens* (1U6G)[35] were used as 3D templates for restraint-based modeling as implemented in the MODELLER program.[36] The overall model was improved enforcing the proper stereochemistry using spatial restraints and CHARMM energy terms, followed by conjugate gradient simulation based on the variable target function method.[36] Ten models were built for the RD2 sequence based on the (m)GenThreader alignment. All models were evaluated with the DOPE potential and the one with the lower global score was selected for explicit solvent MD simulation using GROMACS[37] to check its stability and consistency. The overall and local quality analyses of the final model were assessed by VERIFY3D,[38] PROSA,[39] and VADAR.[40] Three-dimensional structures were displayed, analyzed, and compared using the program COOT.[41]

RESULTS AND DISCUSSION

The 1.5-repeat peptide of PthA shows a TPR-like fold

Secondary structure algorithms predict only α-helices and turns for the repeat domains of PthA proteins. Not

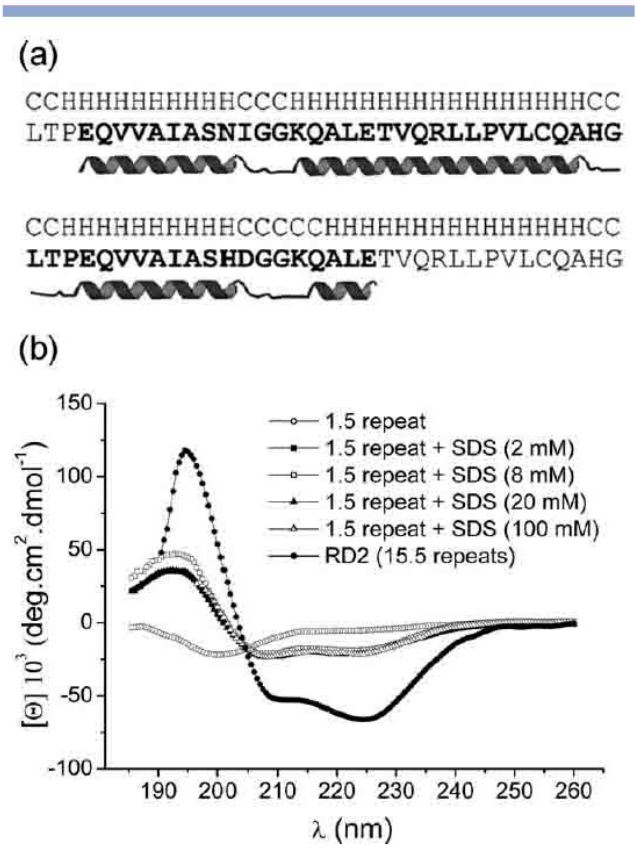

Figure 1

Structural features of the repetitive units of RD2. (**a**) PSIPRED secondary structure prediction of two consecutive repeat units of RD2 showing coils (C) and helices (H). The 1.5-repeat unit peptide used for structural resolution is shown in bold and the secondary structure elements determined by NMR are represented underneath the sequence. (**b**) CD curves of the purified 15.5-repeat domain (5 μ*M*) of PthA2 (RD2) in comparison with that of the 1.5-repeat unit peptide (10 μ*M*) in the presence and absence of different SDS concentrations.

surprisingly, the predictions are almost identical within each of the repeat units, as shown for two consecutive repeats found in the PthA variants [Fig. 1(a)]. The predictions are consistent with the far-UV CD spectrum of the entire 15.5-RD2 showing two minimum peaks at 208 and 222 nm and a positive peak at 193 nm, which indicate that RD2 has high contents of α-helices [Fig. 1(b)].

CD data analysis has indicated that the 34-amino acid peptide corresponding to one repeat unit of PthA is not structured in solution (not shown). Thus, we have used *in silico* 3D modeling algorithms to find a minimal peptide size that possesses the stereochemical requirements for a structured motif, and we observed that the 1.5-repeat peptide is the minimal structural unit of the repeat region of PthA. CD measurements indicated that the 1.5-repeat peptide is partially unfolded in solution; however, addition of 2 m*M* SDS was sufficient to increase the contents of secondary structural elements, particularly α-helices [Fig. 1(b)]. Although SDS has been successfully used to investigate the structure of membrane peptides, it has also been shown to stabilize conformations in peptides with propensity to form α-helices via electrostatic interactions.[42] We therefore used NMR techniques to solve the structure of the 1.5-repeat peptide in the presence of SDS since no experimental 3D model of the repeat unit was yet available.

The 1.5-repeat structure was solved using 982 distance constraints derived from the NOESY spectra. The inter-residue NOEs correlate with the chemical shift index and show a dense pattern of $d\alpha N(i,i+3)$, $d\alpha\beta(i,i+3)$, and $d\alpha N(i,i+4)$ NOEs involving residues V3-I10, Q14-Q20, P24-A29, and V37-G45, indicating that these regions possess an helical fold (Supporting Information Figure 1). Statistics of an ensemble of 20 lower energy structures [Fig. 2(a)] show no significant violations of the molecular geometry parameters (Table I). In addition, the Ramachandran plot analysis shows that all *phi–psi* angles are in allowed regions and the root mean square deviations (RMSD) for the backbone and side chains are within expected values, indicating a good stereochemical quality of the ensemble.

The peptide folds into a helical-bundle structure that is very similar to the TPR topology [Fig. 2(a)], thus corroborating the secondary structure predictions and CD data. In fact, the CD curve of RD2 is quite similar to those of TPR domains with a 222/208 nm ratio of ~1.3, which indicates coiled-coil-like conformations.[43–46] The specific interactions responsible for these conformational states are the presence of a series of hydrogen bonds between the backbone carbonyl (C=O) of residues "*i*" and the backbone amide (NH) of residues "*i* + 4" spanning the helical regions. The secondary structure is also stabilized by hydrophobic interactions that keep the helical regions close together. Preferential spatial arrangement is confirmed by NOE interactions between side chains of the hydrophobic residues V4-S8 with T18-L22 and L16-C27 with L32-H43 [Fig. 2(b)].

Interestingly, the two hypervariable diresidues NI and HD, known to specify preferential interactions with adenine and cytosine in DNA target sites, respectively,[15,16] are structurally close in the NMR structures [Fig. 2(c)]. This would be expected considering that each pair of the variable residues recognizes one nucleotide in a linear fashion, that is, one variable diresidue to one nucleotide in the DNA target sequence.[15,16] We also noticed that the K residues, which typically play a central role in protein–DNA interactions,[47] are located adjacently to the polymorphic diresidues [Fig. 2(c)].

The RD2 has an elongated shape

The *ab initio* molecular shape of RD2 was determined by SAXS analysis and the corresponding profiles of the scattering curve and distance distribution function $p(r)$ are shown in Figure 3(a). SAXS calculations revealed a maximum dimension of 129 Å from the $p(r)$ curve and a gyra-

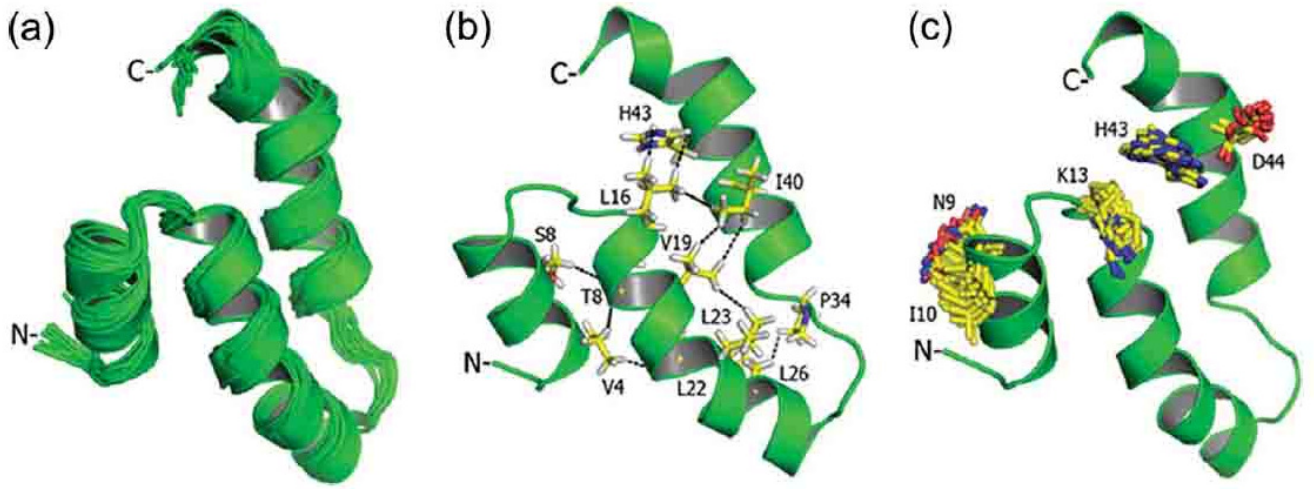

Figure 2

The NMR structure of the 1.5-repeat peptide of PthA. (**a**) Ensemble of the 20 lowest energy structures showing three helices connected by two turns. (**b**) The lowest energy structure showing the hydrophobic interactions between the side chains of residues VVAIA and LLPVL, which stabilize the structure. (**c**) Orientation of the hypervariable diresidues NI and HD relative to the K residue in the peptide structure.

tion radius of 40.82 ± 0.01 Å for the RD2 molecule. These values are in agreement with those obtained from the *in silico* modeling (see below) and indicate that RD2 is a monomer in solution. Analytical size-exclusion chromatography and DLS data also suggest that RD2 is a monomer in solution with a hydrodynamic radius of around 4.5 nm for a sample with low polydispersity index (15%).

The low resolution envelope of RD2 was derived without imposing any constraints [Fig. 3(b)]. Structural alignments of each individual molecular model, obtained by *ab initio* shape reconstruction, showed that they are very similar as indicated by the normalized spatial discrepancy (NSD) values, which are around 0.8. The RD2 model reveals an elongated or extended shape consistent with the notion that the repeat units are structurally arranged in tandem along the repeat region [Fig. 3(b)]. The elongated shape of the RD2 molecule can also be noted from the $p(r)$ curve profile [Fig. 3(a)].

Molecular modeling predicts a TPR-like superhelical structure for RD2

Fold assignment searches for RD2 using PSIPRED[48] returned 10 protein structures with significant scores, all displaying TPR domains. The crystal structures of the yeast importin β1 (PBD code: 2BPT) and human TIP120 (PDB code: 1U6G) were used as templates to generate a restraint-based 3D model of RD2 [Fig. 4(a)]. The model shows good local and global stereochemical properties with a *Z*-score of 6.8. Analyses of the Ramachandran plot indicate that 93% of the RD2 residues are in most favorable regions, 5% are in additional allowed regions and only 2% are in disallowed regions. In addition, local quality analysis assessed by plotting the energies as a function of the amino acid positions shows no positive values thus highlighting the good stereochemical quality of the model and its suitability for structural analysis and comparisons. The NMR structure of the 1.5-repeat peptide superimposes adequately with the RD2 model with an RMSD of 2.7 Å [Fig. 4(b)], thus corroborating the *in silico* model.

The RD2 3D model conserves all the structural features found in TPR-containing proteins encompassing 31 antiparallel α-helices that fold into a superhelical structure [Fig. 4(a)] and is in agreement with previous molecular modeling studies.[49] Furthermore, the superhelix model is consistent with the RD2 CD data showing an average 222/208 nm ratio >1.1, which is typical of coiled-coil regions. The repeat units of RD2 have the same amino acid segment length of TPRs and their superposition indicates that

Table I

Structural Statistics of the 1.5-Repeat Unit Peptide

NOEs	
Total number	982
Short range $\|i-j\| \leq 1$	650
Medium range $1 < \|i-j\| < 5$	156
Long range $\|i-j\| \geq 5$	176
CYANA	
Target function	1.01 ± 0.15
Distance violation >0.20	0
Dihedral angles >5°	0
RMSD	
Backbone (total)	0.72 ± 0.13
Side chain (total)	1.54 ± 0.17
PROCHECK	
Most favorable	84.1%
Additional allowed	15.3%
Generously allowed	0.6%
Not allowed	0.0%

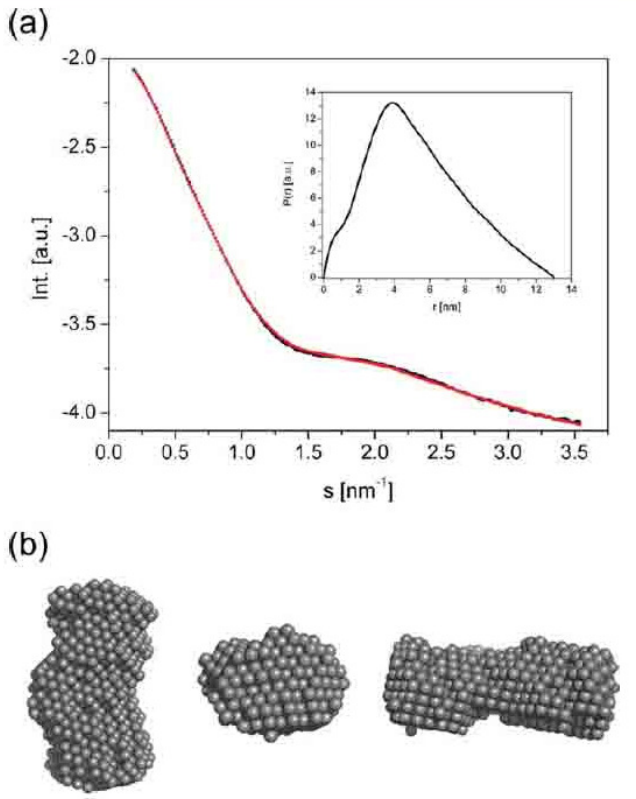

Figure 3

Ab initio shape determination of RD2. (**a**) Experimental SAXS curve and the theoretical fitting of the data (black and red, respectively) using the program GNOM. The inlet shows the pair distance distribution function $p(r)$. (**b**) Different views of the *ab initio* low resolution DAMMIN envelop model of RD2 in solution. The NSD values for a set of 15 DAMMIN and GASBOR models ranged from 0.85 to 0.95 in both calculations.

they are quite similar to one another (RMSD $\leq$ 1.5 Å). As observed in the structure of the 1.5-repeat peptide [Fig. 2(b)], the superhelix is stabilized by contacts of hydrophobic residues across the α-helical faces resulting in a continuous hydrophobic core. Additional polar interactions between close side chains contribute to the protein packing and stabilization. Notably, the *in silico* model fits remarkably well on the SAXS envelope and displays a NSD of 1.25, indicating high-shape complementarity between the *ab initio* envelope and the surface of the theoretical superhelix model [Fig. 4(c)].

TPR domains are a well-known protein scaffolds that mediate interprotein associations and assembly of multiprotein complexes in numerous organisms. The domain was first identified in yeast proteins required for mitosis and RNA synthesis as a degenerated 34-amino acid sequence arranged in tandem repeats.[50,51] Since then, studies have highlighted its diversity of arrangements and functions.[52,53] More recently, it was found that TPR domains can self-interact and promote protein oligomerization.[43,54,55] Thus, the TPR-superhelical structure of RD2 is consistent with the fact that RD2 also self-interacts in two-hybrid assays and associates with a number of citrus proteins, including the TPR domain of TDX.[14]

Conformational changes of RD2 upon interaction with DNA

Although TPR domains have not been reported to bind nucleic acids directly, they are found in numerous DNA and RNA-binding proteins.[51,53,56,57] To gain insights into how the RD2 superhelix could bind DNA with

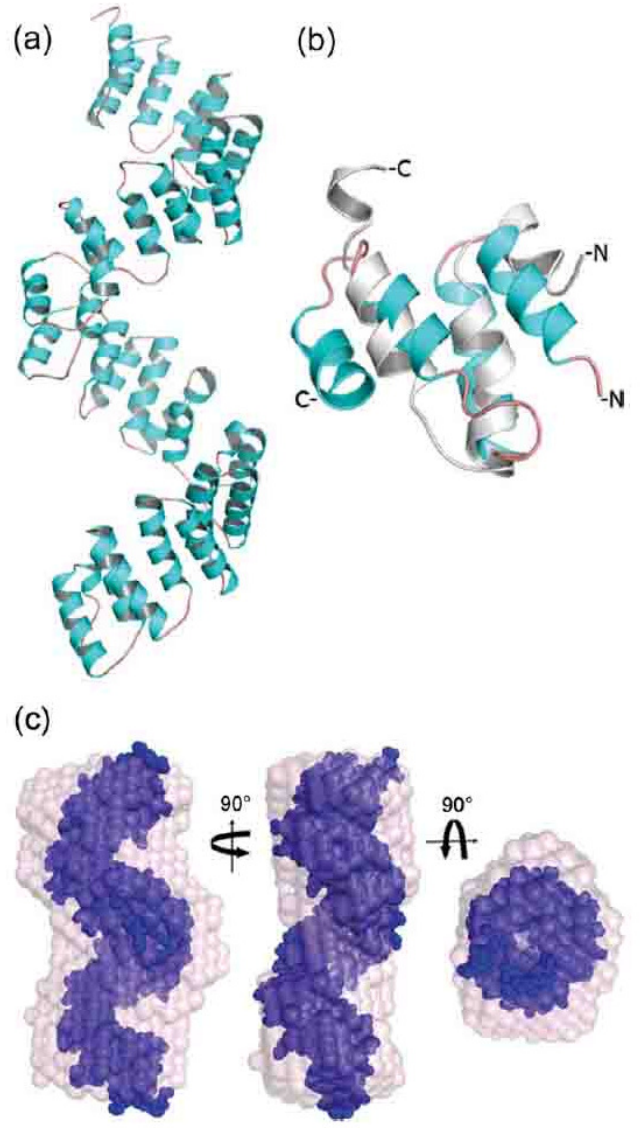

Figure 4

The structural model of RD2. (**a**) A cartoon representation of the TPR-like superhelical structure of RD2 depicting helices in cyan and loops in magenta. (**b**) Superposition of the NMR (gray) and modeled structures (cyan) of the PthA 1.5-repeat unit. (**c**) Orthogonal views illustrating the superposition of the TPR-like superhelical structure of RD2 onto its SAXS envelope.

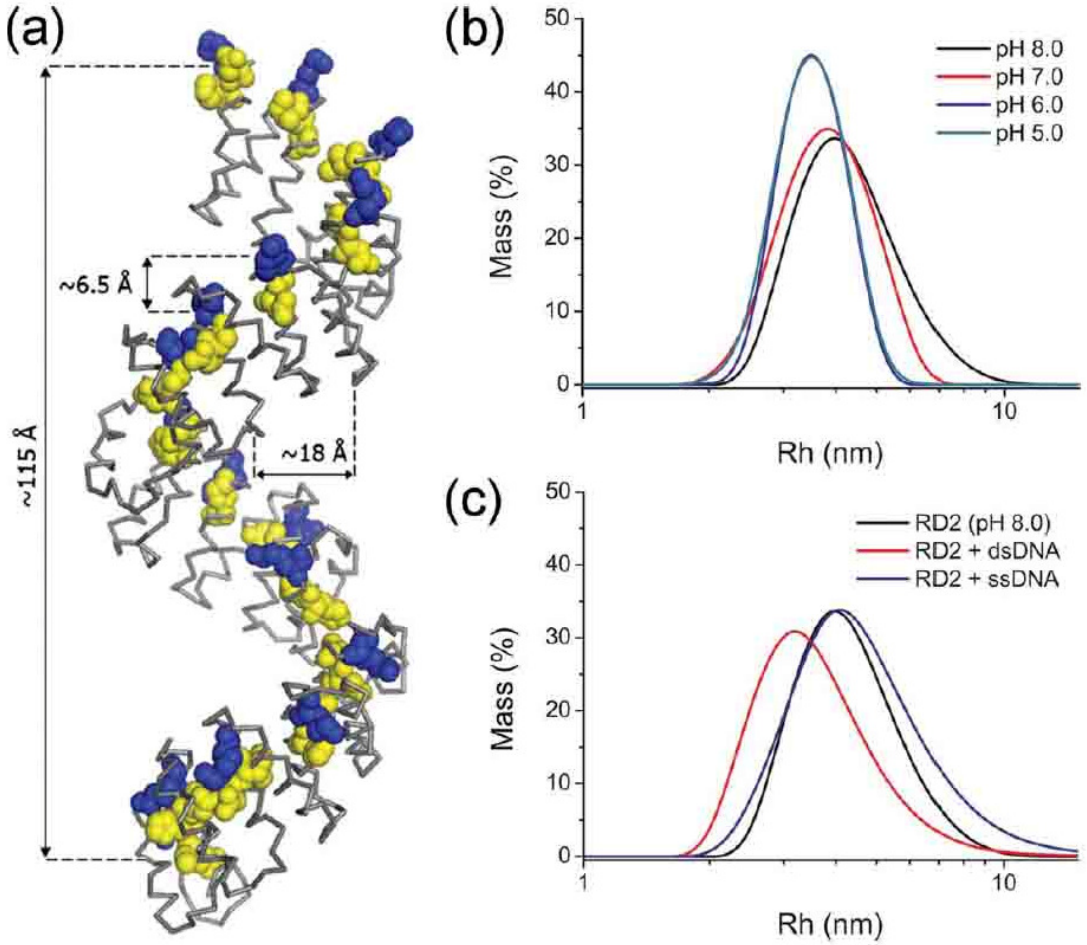

Figure 5

Conformational changes of RD2. (**a**) A ribbon representation of the RD2 TPR-like structure highlighting the relative location of the hypervariable diresidues (yellow spheres) and the lysines (blue spheres). The approximate dimensions of the height, cleft and spacing between two consecutive hypervariable diresidues are shown. DLS curves of RD2 at different pHs (**b**) and after incubation with the single-strand (ss) or double-strand (ds) DNA sequence ACACATTCTAAAATTTATATAAACCCTCATCCATTTCC derived from a citrus PthA-induced promoter (**c**).

sequence specificity, we looked at the spatial distribution of the polymorphic residues at Positions 12 (N or H) and 13 (I, D, or G). As observed in the 1.5-repeat peptide structure [Fig. 2(c)], the NI, HD, and NG pairs are located at the same side of the molecule at the tip of helix1, in close proximity to the K residues [Fig. 5(a)]. Furthermore, it is evident from the RD2 model that the polymorphic diresidues are mostly oriented to the inner face of the superhelix [Fig. 5(a)]. Thus, the only way the polymorphic residues could contact DNA would be the protein embracing the double strand, which is consistent with the fact that ligand binding in TPR proteins predominantly involves the concave surface of the TPR domain.[53] The atomic distances of the superhelix clefts (18 Å wide on average) supports this notion [Fig. 5(a)]. However, both the modeled structure and the SAXS envelop of RD2 have a molecular length of ~115 Å [Fig. 5(a)], which would cover ~34 nucleotides, that is, twice as many nucleotides as predicted to bind RD2.[15] Therefore, to be able to bind a 17-nucleotide target sequence, as predicted by the linear recognition model of one nucleotide per hypervariable diresidues,[15,16] the RD2 protein would have to undergo some degree of compaction, like a coil spring being compressed. Surprisingly, when the hydrodynamic behavior of RD2 was investigated, substantial changes in its hydrodynamic radius (R_h) due to pH changes were found [Fig. 5(b)]. At pH 8.0, the RD2 molecule displays an R_h of 4.5 nm that is consistent with the SAXS measurements done at similar pH. However, at more acidic pH, which might mimic the DNA surface, the R_h values are around 3.5 nm, indicating that RD2 can assume a more compact structure [Fig. 5(b)]. Similarly, RD2 significantly increased its compactness (R_h of 3.6 nm) in the presence of a DNA fragment derived from a citrus promoter upregulated by PthA2 [Fig. 5(c)]. Changes in the hydrodynamic radius was not clearly observed when the corresponding single-strand sense DNA was used [Fig. 5(c)], indicating that compaction preferentially occurs upon double-strand DNA interaction.

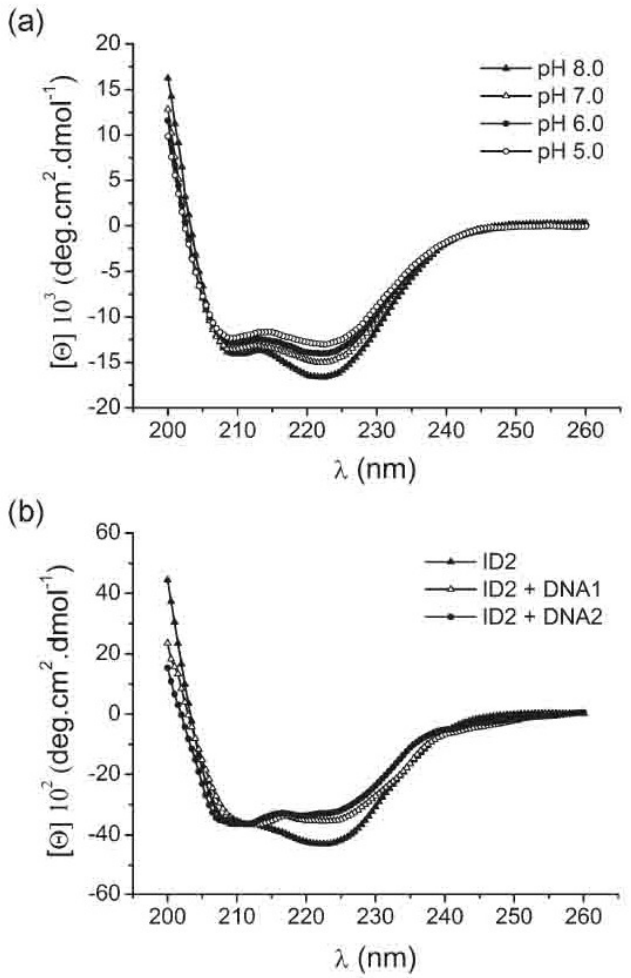

Figure 6

CD spectra of RD2 at different pHs and in the presence of DNA. CD curves of RD2 protein taken at different pHs (**a**) and after incubation with two DNA fragments (**c**), showing major changes in the 222/208 nm ratios at acidic pH and in the presence of DNA. The DNA1 sequence (ACACATTCTAAAATTTAT ATAAACCCTCATCCATTTCC) is derived from a citrus promoter upregulated by PthA2 whereas DNA2 contains the predicted PthA2 binding site[15] (ACAC ATTCTAAAATTTACACACCTCTTTTAATATTTCC).

To further investigate these conformational changes, the secondary structure of RD2 at different pHs and in the presence of DNA was analyzed by CD spectroscopy. At acidic pH, RD2 apparently looses some of its α-helical contents, as judged by the lower intensities of the 208 and 222 nm negative signals at pHs 7.0, 6.0, and 5.0, relative to pH 8.0 [Fig. 6(a)]. However, the 222/208 nm ratio shifted from 1.3 at pH 8.0 to nearly 1.0 at pH 5.0, indicating a decrease in coiled-coil-like conformations at acidic pHs. The 222/208 nm ratio of RD2 also diminished in the presence of DNA. This was observed not only with the citrus promoter fragment but also with a DNA fragment containing the predicted PthA2 binding site[15] [Fig. 6(b)]. Interestingly, it has been shown that a disulfide bond within the TPR region of the barley Sgt1 protein is thought to promote a compaction of the helical bundles.[43] The reduced Sgt1 has a CD 222/208 nm ratio of ~1.2, whereas oxidized Sgt1 has a ratio of ~0.9,[43] indicating that compaction of the TPR helical bundles is associated with a decrease in its coiled-coil-like conformation.

The repeat units of RD2 are similar to PPR motifs

As TPR domains show no obvious sequence similarities to the repeat units of RD2 and so far have not been reported to bind DNA directly, we searched for TPR-related motifs with known nucleic acid binding properties. Surprisingly, we found that the consensus sequence of PPR motifs[58,59] is very similar to that of the repeat units of RD2 (see Fig. 7). In addition, BLAST searches using the RD2 sequence as query also identified a number of Arabidopsis (Q9MA95.2, NP_187175.1) and rice (Os02g0555100, OsJ_07125, OsJ_12287) proteins containing PPR motifs.

PPRs are formed by 2–26 tandem arrays of a degenerate 35 amino acid motif with an average of 9–12 motifs per protein.[17,58] PPR proteins have been associated with the transcription and translational machineries, playing roles in mRNA stabilization and RNA editing.[17,18,60] Interestingly, some of the citrus proteins that were isolated in a two-hybrid screening as interactors of PthAs are associated with RNA stabilization.[14] Although no PPR structures are known, tandem PPR motifs are predicted to fold into a superhelical structure just like the TPR superhelix.[18,58] Accordingly, the CD spectrum of maize PPR5 resembles that of RD2[17] and a structural model of PPRs superposes well to the RD2 superhelix (results not shown). Thus, the structure of RD2 proposed here is consistent with the notion that nucleic acid-binding PPR motifs display a TPR-like fold. Although PPR proteins are almost absent in prokaryotes, the fact that they are predominantly found in plant mitochondria and chloroplasts[61] raises the possibility that PthAs and related type III effectors might have evolved from a common PPR ancestor protein.

```
ETVQRLLPVLCQAHGLTPEQVVAIASHDG-GKQAL RD2 rep2
ETVQRLLPVLCQAHGLTPDQVVAIASHDG-GKQAL RD2 rep6
ETVQRLLPVLCQAHGLTPQQVVAIASNGG-GKQAL RD2 rep8
EEA..LY..M....G..PN..TYNALINAYAK.G. PPR cons.
*  .  *   :    *  *:  .  *  .. .* .
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Figure 7

The repeat units of RD2 are similar to PPR motifs. Protein sequence alignment between three polymorphic repeat units of RD2 and the consensus sequence from 1303 pentatricopeptide (PPR) motifs[57] performed by ClustalW2 (EMBL-EBI). Identical residues are shaded and indicated by asterisks, whereas similar residues are indicated by single or double dots. Dots in the PPR consensus sequence represent the most variable residues in PPR motifs.

In conclusion, this work provides the first experimental structural data of the PthA repeat domain. Both the NMR structure of the 1.5-repeat peptide and the SAXS envelope of RD2 corroborate the theoretical model that predicts a TPR-superhelical structure for RD2. Moreover, DLS and CD studies show that RD2 undergoes conformational rearrangements upon DNA interaction thus supporting the idea that the protein embraces the DNA with differential compactness as to cover a variable number of nucleotides.

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