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FERNANDA NUNES PERON

ANÁLISE TRANSCRIPTÔMICA DA INTERAÇÃO DO ABACAXIZEIRO COM O *Pineapple mealybug wilt-associated virus*

VITÓRIA – ES

2018

RENORBIO

Programa de Pós-Graduação em Biotecnologia

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Tese apresentada ao Programa de Pós-Graduação em Biotecnologia da Rede Nordeste de Biotecnologia (RENORBIO) e Universidade Federal do Espírito Santo (UFES) como requisito parcial para obtenção do título de Doutora em Biotecnologia.

Orientadora:Prof[a].Dr[a].Patricia Machado Bueno Fernandes

Coorientador: Prof. Dr. José Aires Ventura

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A minha família, dedico.

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RESUMO

PERON, F.N. **Análise transcriptômica da interação do abacaxizeiro com *Pineapple mealybug wilt associated vírus*.** 2018. 118f. Tese (Doutorado em Biotecnologia) – Programa de Pós-Graduação em Biotecnologia, UFES, Espírito Santo. Brasil.

Ananas comosus var *comosus* é uma fruteira de grande valor econômico e nutricional. A produtividade da cultura do abacaxi é influenciada pela virose murcha do abacaxizeiro (MWP, do inglês *mealybug wilt pineapple*). Essa doença é causada pelo *Pineapple mealybug wilt-associated virus* (PMWaV) sendo as variantes PMWaV-1 e PMWaV-2 associadas aos sintomas da doença. Os sintomas decorrem da morte das raízes seguido de murcha e descoloração das folhas com avermelhamento e consequente alteração na floração e frutificação. O controle da disseminação da doença pela remoção das plantas sintomáticas não é satisfatório especialmente porque plantas assintomáticas infectadas servem de fonte de inóculo do vírus através de mudas infectadas. O transcriptoma diferencial entre plantas infectadas sintomáticas e assintomáticas foi avaliado por RNA-seq, ferramentas de bioinfomática e RT-qPCR para propor uma compreensão da patogênese do PMWaV em condições de campo. Adicionalmente proteínas foram confirmadas por análise de espectrometria de massa. Com base no genoma de referência de *Ananas comosus*, foram identificados 16.097 genes expressos sendo 268 reprimidos e 122 induzidos. A performace do RNA-seq foi confirmada para 14 genes direrencialmente expressos (DEGs) com uma correlação de Pearson satisfatória (R = 0,79). Análises de classificação funcional e enriquecimento revelaram indução de genes envolvidos na regulação da floração enquanto que os genes reprimidos foram predominantemente relacionados aos mecanismos de defesa a estresse abiótico e biótico. Entre eles, alguns fatores de transcrição (FT) *WRKY*s e *MYB*s; *PR*s; *HSP*s; *AQP*s e genes que codificam enzimas removedoras de ROS e transportadores de Cobre, Cálcio e Zinco foram reprimidos. Por outro lado, a expressão de genes responsivos a auxina foram positivamente regulados por *ARFs*. Observamos regulação hormonal mediada pela inibição da biossíntese de jasmonato (JA), indução da biossíntese de etileno (ET) e indução da expressão de genes responsivos a auxina e que foi relacionada ao desenvolvimento de sintomas. Uma rede de interação proteina-proteina foi predita

permitindo a visualização da interação dos produtos gênicos dos DEGs com agrupamento de chaperonas. A inibição da expressão dos genes que codificam as chaperonas ERDJ3B, BiP2 e RTM2 foi confirmada em plantas sintomáticas. Além disso, uma significativa correlação negativa (R = -0,715) entre os níveis de transcritos de *RTM2* e PMWaV-2 foi identificada. Como a expressão desse vírus é predominante nas plantas sintomáticas, propomos *RTM2* como um provável gene associado ao controle do deslocamento de PMWaV-2 via floema. Além disso, uma proteína HSP20 foi identificada somente nas amostras de plantas assintomáticas reforçando a hipótese do envolvimento de uma HSP20 no controle da infecção. Foram também identificadas as proteínas HSP70, CaM e CRT. Estas proteínas tiveram a expressão gênica reprimida nas plantas sintomáticas apontando para o estresse no retículo endoplasmático (RE) como reposta a infecção pelo PMWaV. Como os vírus necessitam da maquinaria celular, admiti-se que as plantas de abacaxi reprimam a expressão de chaperonas residentes no RE na via UPR (do inglês, *unfolfed protein response*) como estratégia para limitar o patógeno no sítio de infecção. Contudo, com a inbição da via SAR (do inglês, *systemic acquired resistance*) e da expressão de *RTM2*, possíveis barreiras ao deslocamento de PMWaV-2 foram suprimidas. Além disso, a expressão de IRE1 revela a ativação da UPR para a morte celular por apoptose. Assim, surpreendentemente conclui-se que a infecção por PMWaV-2 desencadeia a resposta hipersensitiva através da UPR além de modular a expressão de genes R. Diante do exposto, esta tese contribui para a revisão dos métodos adotados para o manejo de MWP tanto a nível dos tratos culturais como a nível de melhoramento genético do abacaxi.

Palavras chave: Murcha, PMWaV-2, RNA-seq, *unfolded protein reponse*, *RTM2.*

ABSTRACT

PERON, F.N. **Transcriptomic analysis of the interaction between pineapple and *Pineapple mealybug wilt associated vírus*.** 2018. 118f. Thesis (Doctoral in Biotechnology) – Postgraduation Biotechnological Programme, UFES, Espírito Santo. Brazil.

Ananas comosus var *comosus* is a fruit of great economic and nutritional value worldwide. The productivity of the pineapple crop is influenced by mealybug wilt of pineapple (MWP). This disease is caused by *Pineapple mealybug wilt-associated virus* (PMWaV), the PMWaV-1 and PMWaV-2 variants are associated with the symptoms of the disease. The symptoms derive from the atrophy of the roots followed by wilting and discoloration of the leaves with redness and consequent deficiency in fruiting. The control of the disease occurs by the removal of symptomatic plants is not satisfactory especially since infected asymptomatic plants serve as a source of virus dispersion through their seedlings. Therefore, in this work, the differential transcriptome between symptomatic and asymptomatic infected plants was evaluated by RNA-seq, bioinformatic tools and RT-qPCR to propose an understanding of the pathogenesis of MWP under field conditions. Additionally, the proteins were confirmed by mass spectrometry analysis. Based on the reference genome of Ananas comosus, 16,097 expressed genes were identified, 268 repressed and 122 induced. The performance of RNA-seq was confirmed for 14 expressively expressed genes (DEGs) with a satisfactory Pearson correlation (R = 0.788). Functional classification and enrichment analysis revealed induction of genes involved in the regulation of flowering while repressed genes were predominantly related to the defense mechanisms of abiotic and biotic stress. Among them, some transcription factors (FT) *WRKY*s and *MYB*s, *PR*s, *HSP*s, *AQP*s and genes encoding ROS-removing enzymes, and Copper, Calcium and Zinc transporters were repressed. On the other hand, the expression of auxin responsive genes was positively regulated by *ARFs*. We observed hormonal regulation mediated by inhibition of jasmonate biosynthesis (JA), induction of ethylene biosynthesis (ET) and induction of the expression of genes responsive to auxin, what was related to the development of symptoms. A protein-protein interaction network was predicted allowing the visualization of the interaction of the gene products of the DEGs.

Therefore, it was possible to observe the grouping of chaperones in the center of the network. We confirmed the inhibition of expression of the genes encoding the ERDJ3B, BiP2 and RTM2 chaperones in symptomatic plants and identified a significant negative correlation (R = -0.715) between the levels of *RTM2* and PMWaV-2 transcripts. As the expression of this virus is predominant in symptomatic plants, we propose *RTM2* as a probable gene associated with PMWaV-2 dispersion control. In addition, an HSP20 protein was identified only in asymptomatic plant samples reinforcing the hypothesis of HSP20 involvement in infection control. In the same way, we identified the HSP70, CaM and CRT proteins. These proteins had the repressed gene expression in the symptomatic plants pointing to stress in the endoplasmic reticulum (ER) as a response to PMWaV infection. Because viruses require the cellular machinery, we believe that pineapple plants suppress the expression of resident chaperones in the UPR (unfolfed protein response) as a strategy to limit the pathogen at the site of infection. However, with the introduction of SAR (systemic acquired resistance) and RTM2 expression, possible barriers to the displacement of PMWaV-2 were suppressed. In addition, the IRE1 expression reveals the activation of UPR to cell death by apoptosis. Thus, it is surprisingly concluded that PMWaV-2 infection elicits a hypersensitive response through UPR, in addition to modulating R gene expression.In view of the above, this thesis contributes to the revision of the methods adopted for the management of MWP both in cultural practices and the level of genetic improvement of pineapple.

Keywords: Wilt, PMWaV-2, RNA-seq, unfolded protein response, *RTM2*.

LISTA DE ABREVIATURAS E SIGLAS

BIP - Proteína de ligação a imunoglobulina (do inglês, *Binding Immunoglobulin Protein*)
BTH - benzotiadiazol-éster metílico
CAM - Metabolismo ácido das crasuláceas (do inglês, *Crassulacean Acid Metabolism*)
DEGs - Genes diferencialmente expressos (do inglês, *Differentially expressed genes*)
ET - Etileno
ERAD - Degradação de proteína associada ao retículo endoplasmático (do inglês, E*ndoplasmic-Reticulum-Associated Protein Degradation*)
FAO - Food and Agriculture Organization of the United Nations
FT - Fator de transcrição
GLRaV - *Grapevine leafroll-associated vírus*
HR - Resposta hipersensitiva (do inglês, *Hypersensitive Response*)
MWP - Murcha do abacaxizeiro (do inglês, *Mealybug wilt of Pineapple*)
NBS-LRR - Proteínas ricas em leucina no sítio de ligação a nucleotídeo (do inglês, *Nucleotide-Binding Site Leucine-Rich Repeat protein*)
NGS - Sequenciamento de nova geração (do inglês, *Next Generation Sequencing*)
PMWaV - *Pineapple mealybug wilt-associated vírus*
PRs - Proteínas relacionadas à patogênese (do inglês, *Pathogenesis Related Proteins*)
RISC - Complexo de silenciamento induzido pelo RNA (do inglês, *RNA-Induced Silencing Complex*)
RNA-seq - Sequenciamento do RNA
ROS/EROs - Espécies reativas de oxigênio (do inglês, *Reactive Oxygen Species*)
RTM - *Restricted TEV Movement*
SAR - Resistência sistêmica adquirida (do inglês, S*ystemic Adquirid Resistance*)
TEV - *Tobacco etch virus*
UPR - Resposta a proteína mal enovelada (do inglês, U*nfolded Protein Response*)
UPS - Sistema ubiquitina - proteassoma (do inglês, *Ubiquitin-Proteasome System*)

SUMÁRIO

1 INTRODUÇÃO

Frutos do abacaxi *Ananas comosus* var *comosus*, são consumidos mundialmente em razão de suas vantagens nutricionais e medicinais (COSTA et al., 2014; RAMLI; AZNAN; ILLIAS, 2017). Além disso, apresentam potencial biotecnológico com valor agregado a extração de suas enzimas proteolíticas bromelinas e ao uso da planta como artefato ornamental (COSTA et al., 2014). A produção mundial dos frutos de abacaxi concentra-se em países asiáticos e americanos sendo a Costa Rica, o Brasil e Filipinas os maiores produtores. Em 2016, a produção mundial do abacaxi foi de 25,8 milhões de toneladas o que movimentou acima de 25 bilhões de dólares (FAO, 2018).

Entraves à produção do abacaxi incluem as doenças que limitam sua produção, entre elas a murcha do abacaxizeiro (MWP, do inglês *Mealybug wilt of pineapple*). Esta doença pode levar a perdas significativas na produção mundial na ordem de 80% (GUNASINGHE, 1989; MAYO, 2002; VENTURA; ZAMBOLIM, 2002), afetando regiões produtoras como Hawaii, Cuba, Austrália, Brasil e Tailândia (SETHER et al., 2005a, 2009; SUBERE et al., 2011; VENTURA; ZAMBOLIM, 2002).

Os sintomas da doença ocorrem à partir da morte das raízes, seguidos de murcha e descoloração gradual das folhas com intensa cor vermelha, que curvam-se em direção ao solo e apresentam ressecamento das pontas. Plantas infectadas apresentam dificuldade na floração e frutificação, produzindo frutos atrofiados e murchos, impróprios ao consumo ou à industrialização (VENTURA; ZAMBOLIM, 2002a). Causada pelo complexo *Pineapple mealybug wilt-associated virus* (PMWaV) transmitido pelas cochonilhas *Dysmicoccus brevipes* e *Dysmicoccus neobrevipes*, quando descrita em 1933 no Havai, a MWP foi inicialmente associada a possíveis toxinas introduzidas pelas cochonilhas, razão pela qual recebeu o nome de *Mealybug wilt of pineapple* (CARTER, 1939). Após anos de estudos ficou evidenciado a necessidade da presença do PMWaV para que os sintomas acontecessem (GUNASINGHE, 1989; SETHER et al., 2005).

Os estudos para compreender a etiologia de MWP ainda estão em andamento e tem sido direcionados a confirmação do agente causal e sua interação molecular com a planta. Adicionalmente estudos para o melhoramento vegetal do abacaxi com base no estudo do genoma do patógeno e limpeza clonal foram realizados (MELZER et al., 2001; PEREZ et al., 2006; SETHER et al., 2005). Além disso, recentemente foram avaliados fatores de patogenicidade dos vírus PMWaV-1 e PMWaV-2, tendo sido demonstrado a atividade supressora do silenciamento do ácido ribonucleico (RNA) em *Nicotiana benthamiana*. Foi sugerido que o vírus PMWaV-2 estaria envolvido na etiologia de MWP por codificar muitas proteínas com atividade supressora local e sistêmica enquanto que o vírus PMWaV-1 codifica apenas uma e com ação apenas sistêmica (DEY et al., 2015). O PMWaV-2 é na maioria dos países relatado como agente causal da MWP a exceção na Austrália (ALBRECHT; BOWMAN, 2008; BORROTO-FERNANDEZ; COSTA; LAIMER, 2007; GAMBLEY et al., 2008; SETHER et al., 2005a; SETHER; HU, 2002; SHEN et al., 2009). Além disso, plantas assintomáticas infectadas permanecem no campo servindo como fonte de inoculo através de suas mudas. Todavia, a sobrevivência destas plantas ao lado das plantas sintomáticas revela mecanismos de tolerância no abacaxi que podem servir de estudo para o entendimento da defesa da planta.

Até o presente momento não foram relatados estudos que elucidem a resposta molecular específica da planta ao patógeno em razão dos sintomas atribuídos a doença MWP. Uma alternativa seria o estudo do transcriptoma diferencial de plantas de abacaxi infectadas assintomáticas e sintomáticas, podendo direcionar o entendimento do progresso da virose e viabilizar estudos de melhoramento vegetal que favoreçam uma abacaxicultura mais produtiva, resistente e ou tolerante ao PMWaV.

Diferentes ferramentas moleculares permitem o estudo parcial do transcriptoma contudo, com o avanço das tecnologias de sequenciamento de nova geração (NGS), o sequenciamento do mRNA (RNA-seq) ampliou a cobertura experimental e acelerou pesquisas de expressão gênica. O transcriptoma do abacaxi vem sendo explorado por análise *de novo* para compreender a maturação do fruto em *A. comosus* var *comosus* (ONG; VOO; KUMAR, 2012) e para identificar genes de resposta a indução por um precursor do etileno, o ethephon (LIU; FAN, 2016). Em

2015, o genoma de *A. comosus* foi sequenciado e organizado para elaborar um modelo de estudo do metabolismo ácido das crassuláceas (CAM) (MING et al., 2015; ZHANG; LIU; MING, 2014). A identificação de genes expressos em estudo de transcriptomas de abacaxi se tornou possível com o genoma de referência sequenciado. O sucesso da identificação *in silico* destes genes requer a seleção e manipulação de diversas ferramentas de bioinformática que reduzam a identificação de falsos positivos e permitam propor genes candidatos a estudos de melhoramento vegetal. O presente estudo objetiva identificar genes envolvidos na patogênese e sintomatologia da murcha em condições de campo, através do RNAseq, podendo oferecer *insights* biotecnológicos mais condizentes com a realidade da cultura do abacaxi.

2 OBJETIVO GERAL

Identificar genes do abacaxizeiro envolvidos na interação planta-patógeno PMWaV e direcionar pesquisas de melhoramento vegetal.

2.1 OBJETIVOS ESPECÍFICOS

- Selecionar triplicatas biológicas de abacaxizeiros ‘Smooth Cayenne’, com e sem sintomas de MWP, e caracterizá-las com base na prevalência dos vírus PMWaV-1, PMWaV-2 e PMWaV-3 por qRT-PCR;

- Sequenciar o transcriptoma dos abacaxizeiros selecionados usando o sistema Illumina HiSeq2000;

- Identificar os genes relacionados ao transcriptoma através do mapeamento das sequências obtidas contra o genoma de referência *Ananas comosus* v3;

- Detectar genes diferencialmente expressos (DEGs) entre os grupos de abacaxizeiros sintomáticos e assintomáticos;

- Obter a anotação funcional dos DEGs e identificar os genes agrupados nas categorias funcionais enriquecidos no Gene Ontology;

- Avaliar o enriquecimento funcional dos genes induzidos e dos genes reprimidos para sugerir genes envolvidos no desenvolvimento da doença;

- Obter uma rede predita de interação de proteínas para visualizar centralidade dos DEGs;

- Propor genes envolvidos na regulação transcripcional associados à MWP.

3 REVISÃO DE LITERATURA

3.1 IMPORTÂNCIA DA PRODUÇÃO DO ABACAXI

O abacaxizeiro é um planta monocotiledônea pertencente a família Bromeliaceae, sub-família Bromelioidae, que agrupa cultivares comerciais da espécie *A.comosus* var *comosus* (PY; LACOEUILHE; TEISSON, 1984). Seu fruto é de grande interesse nutricional em razão de sua polpa comestível e rica em açúcares, ácidos orgânicos, vitamina A, B1 e C, além de minerais e subprodutos de interesse medicinal (MANICA, 2000). Além disso, trata-se de uma planta de metabolismo ácido das crassuláceas (CAM) sendo de grande interesse a compreensão dos mecanismos de resistência a seca e ao calor, e a maturação do fruto (ONG; VOO; KUMAR, 2012; ZHANG; LIU; MING, 2014).

A produção mundial do abacaxi concentra-se em países da Ásia e da América, sendo a Costa Rica, o Brazil e Filipinas os maiores produtores. Como importadores prioritariamente os Estados Unidos da América (EUA) seguido da Holanda e Alemanha (FAO, 2018). No período de 2009 a 2016, a produção mundial do abacaxi aumentou de aproximadamente 20 para 25,8 milhões (M) de toneladas (T). Nesse período o Brasil se manteve entre os 3 principais produtores tendo ocupado a posição de maior produtor no ano de 2009 quando chegou a exportar 19.818 T com a produção de aproximandamente 2,2 MT. Contudo, nos anos seguintes, o volume exportado apresentou queda significativa para 3.014 T no ano de 2016 embora a produção tenha se mantido estável com pequenos aumentos anuais. De 2009 para 2016 registrou-se um aumento de 14% de área colhida resultando em um aumento de 22% na produção e consequente valorização. Embora o Brasil ocupe atualmente a segunda posição no ranking dos países produtores, a produtividade é 50% inferior a alcançada na Costa Rica (FAO, 2018).

Dentre os fatores que interferem na produtividade e qualidade do abacaxi para aceitação tanto do mercado interno como externo, a adequação da produção e manejo às exigências fitossanitárias é determinante. Sendo assim, o investimento no aumento da produtividade em conformidade com as demandas do mercado

internacional pode elevar a taxa de exportação contribuindo para o PIB nacional além de otimizar a produção.

Entraves à produção desta fruteira resumem-se na dificuldade em manter a cultura nas melhores condições fitossanitárias. Dentre as doenças que podem afetar a plantação, a murcha do abacaxizeiro (MWP) é uma virose que limita o manejo em razão de sua dispersão discreta por meio das mudas. Sendo o abacaxizeiro propagado vegetativamente, as mudas são selecionadas com base na condição fitossanitária das plantas mãe (MANICA, 2000). Como a presença dos vírus que causam MWP demoram a induzir sintomas nas plantas, mudas são retiradas de plantas assintomáticas, porém infectadas, dispersando os vírus para outras plantações levando a perdas de até 80% da produção (GUNASINGHE, 1989; MAYO, 2002).

3.2 MURCHA DO ABACAXIZEIRO

A MWP é uma doença que ocorre na plantação de abacaxi (Figura 1A) causando sintomas que se iniciam pelo ressecamento das raízes. Posteriormente, ocorre a murcha da planta e descoloração gradual das folhas que ficam avermelhadas com bordas contorcidas no sentido abaxial e apresentam as pontas em epinastia e secas (Figura 1B). Plantas infectadas dificilmente chegam a frutificar ou seus frutos ficam atrofiados, murchos e impróprios ao consumo *in natura* ou à industrialização (SANCHES, 2005; VENTURA; ZAMBOLIM, 2002).

Figura 1 Plantação de abacaxizeiros dispostos em linhas duplas (A). Plantas ‘Smooth Cayenne’ com sintomas de MWP (B). Fonte: José Aires Ventura (2009).

3.2.1 Etiologia da Murcha do Abacaxizeiro

O estudo da etiologia de MWP teve início no Havaí, EUA, em 1939, quando Carter atribuiu às cochonilhas, *Dysmicoccus brevipes,* presentes na plantação de abacaxi a indução dos sintomas observados (Figura 2A-B). Durante anos, pensou-se que as cochonilhas introduziam fitotoxinas no abacaxi ao se alimentarem da seiva (CARTER, 1939). Porém, em 1961, Carter e Ito observaram que plantas assintomáticas estavam disseminando a doença levantando a hipótese de um agente infeccioso (CARTER, 1961). Só no final dos anos 80, vírus filamentosos com RNA fita simples foram purificadas a partir de abacaxizeiros com sintomas de murcha. Foi então proposta a etiologia viral sendo o patógeno descrito como *Pineapple mealybug wilt-associated virus* (Figura 3) (GUNASINGHE, 1989).

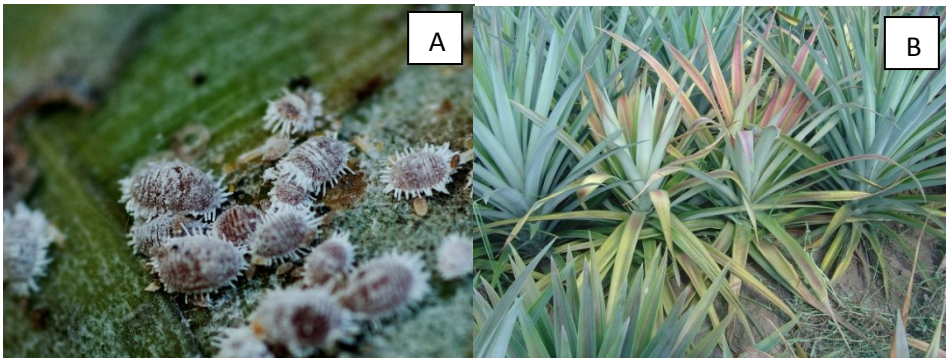

Figura 2 Visão ampliada da infestação de cochonilhas (1mm de comprimento) em folhas de abacaxizeiro (A). Plantas com e sem sintomas de MWP (B).

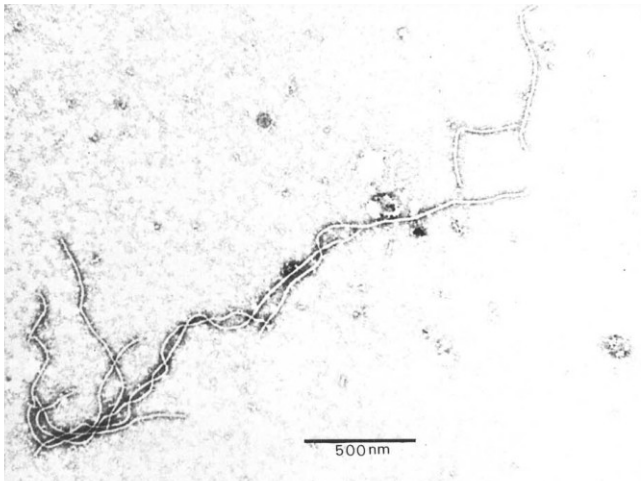

Figura 3 Micrografia eletrônica de partículas virais de PMWaV. Fonte: GUNASINGHE; GERMAN, 1989.

Descoberta a presença de vírus associados aos sintomas de MWP, a caracterização se tornou o próximo passo para entender a patogênese. Por meio de microscopia eletrônica imunoenzimática (ISEM), anticorpos monoclonais e do sequenciamento de porções substanciais do genoma, em 1996, dois vírus foram identificados, *Pineapple mealybug wilt-associated virus 1* (PMWaV-1) e *Pineapple mealybug wilt-associated virus 2* (PMWaV-2), agrupados taxonomicamente com *Grapevine leafroll-associated vírus 3* (GLRaV-3) no gênero *Ampelovirus* (MELZER et al., 2001).

Mais tarde, foram propostos novos vírus do complexo PMWaV com base na análise comparativa de sequências conservadas de aminoácidos de representantes do gênero *Ampelovírus*. Os novos vírus foram denominados *Pineapple mealybug wilt-associated virus 3* (PMWaV-3) e *Pineapple mealybug wilt-associated virus 4* (PMWaV-4) (SETHER et al., 2005a, 2009). Em adição ao complexo PMWaV, em 2008, foi proposto o *Pineapple mealybug wilt-associated virus 5* (PMWaV-5) (GAMBLEY et al., 2008).

Para compreender quais os vírus estão associados aos sintomas da doença, ou se apenas um deles seria o agente etiológico, diversos experimentos de detecção molecular foram realizados em abacaxizeiros cultivados em países de regiões distintas. Inicialmente detectado no Havaí, PMWaV-2 é o vírus mais amplamente relatado como agente etiológico tendo sido detectado em todas as plantas sintomáticas. Os vírus PMWaV-1 e PMWaV-3 são relatados como vírus associados e que poderiam atuar sinergicamente com o PMWaV-2 mas não são atribuídos a indução dos sintomas. Essa relação do vírus PMWaV-2 com os sintomas foi validada em plantas do Havaí, Brasil, Cuba e Taiwan (BORROTO-FERNANDEZ; COSTA; LAIMER, 2007; PERON; FERNANDES; VENTURA, 2009; SETHER et al., 2005). Contudo, na Austrália, a indução dos sintomas foi atribuída aos vírus PMWaV-1 e PMWaV-3 (GAMBLEY et al., 2008).

Adicionalmente, foi demonstrada a participação das cochonilhas *Dysmicoccus brevipes* e *Dysmicoccus neobrevipes* na transmissão dos vírus (SETHER; ULLMAN; HU, 1998) e na determinação dos sintomas de MWP. Plantas infectadas por PMWaV-2 na presença destes insetos desenvolveram sintomas severos da doença enquanto que em plantas infectadas por PMWaV-1 o mesmo não foi observado.

Também foi sugerida a associação com badnavírus tendo sido avaliado o efeito da co-infecção de badnavírus e cada PMWaV na evolução dos sintomas. Sintomas severos foram observados na presença de PMWaV-2, badnavírus e cochonilhas (SETHER; HU, 2002).

Recentemente, fatores de patogenicidade dos vírus PMWaV-1 e PMWaV-2 com atividade supressora do silenciamento do RNA da planta hospedeira foram avaliados em *Nicotiana benthamiana*. Ambos os vírus codificam proteínas com ação sistêmica, contudo, somente PMWaV-2 também codifica proteínas com ação local. Além disso, PMWaV-1 codifica somente p61 enquanto que PMWaV-2 codifica p20 e CP com ação local e sistêmica além de p22 e CPd com ação sistêmica. A expressão de multi proteínas supressoras do silenciamento pelo vírus PMWaV-2 confirma o potencial etiológico deste patógeno (DEY et al., 2015).

3.2.2 *Pineapple mealybug wilt-associated virus - PMWaV*

Os vírus designados como *Pineapple mealybug wilt-associated virus* (PMWaV) são agrupados no gênero *Ampelovírus* da família *Closteroviridae*. Neste gênero também são classificados os vírus *Grapevine leafroll associated virus 3* (GLRaV-3), *Grapevine leafroll associated virus 1* (GLRaV-1) e *Plum bark necrosis stem pitting-associated virus* (PBNSPaV) (MARTINELLI et al., 2012; SETHER et al., 2009). Os ampelovirus apresentam um genoma de RNA positivo de filamento único estimado entre 16,9 e 17,9 Kb. Não apresentam uma terminação poly A na extremidade 3' e possivelmente apresentam um nucleotídeo metilado na extremidade 5'. Seu corpo é constituído pela proteína do capsídeo (CP) e pela proteína capsidial duplicada (CPd). Quando no interior da célula, são imediatamente transcritos pelos ribossomos. As proteínas da ORF1a e ORF1ab, que contém a RdRp, são traduzidas por deslocamento de quadros ribossômicos enquanto que as outras ORFs são traduzidas à partir de RNAs subgenômicos (MARTINELLI et al., 2012; VIRALZONE, 2018).

O primeiro genoma de PMWaV sequenciado foi o do vírus PMWaV-2 tendo o seu genoma de aproximadamente 14,9 Kb parcialmente organizado em 10 *open reading*

frames (ORFs). A partir da homologia das ORFs com as de outros patógenos correlatos, foram preditas as funções. A primeira ORF (ORF1a), possivelmente codifica poliproteínas de aproximadamente 204 KDa, abrangendo protease (PRO), metiltransferase (MTR) e o domínio da helicase (HEL) (Figura 4). Sua CP e CPd são estimadas em 33,8 KDa e 55,8KDa respectivamente (MELZER et al., 2001).

Da mesma forma, em 2008, foi sequenciado e organizado o genoma do PMWaV-1 estimado em 13,1 Kb de comprimento. Diferentemente de PMWaV-2, este vírus não apresenta CPd e a massa molecular da CP é estimada em 28KDa. Por se tratar de um vírus do complexo PMWaV e ser transmitido por cochonilhas, PMWaV-1 foi agrupado no mesmo gênero com PMWaV-2 embora seu genoma seja mais estritamente relacionado a PBNSPaV (Figura 4) (MELZER et al., 2008).

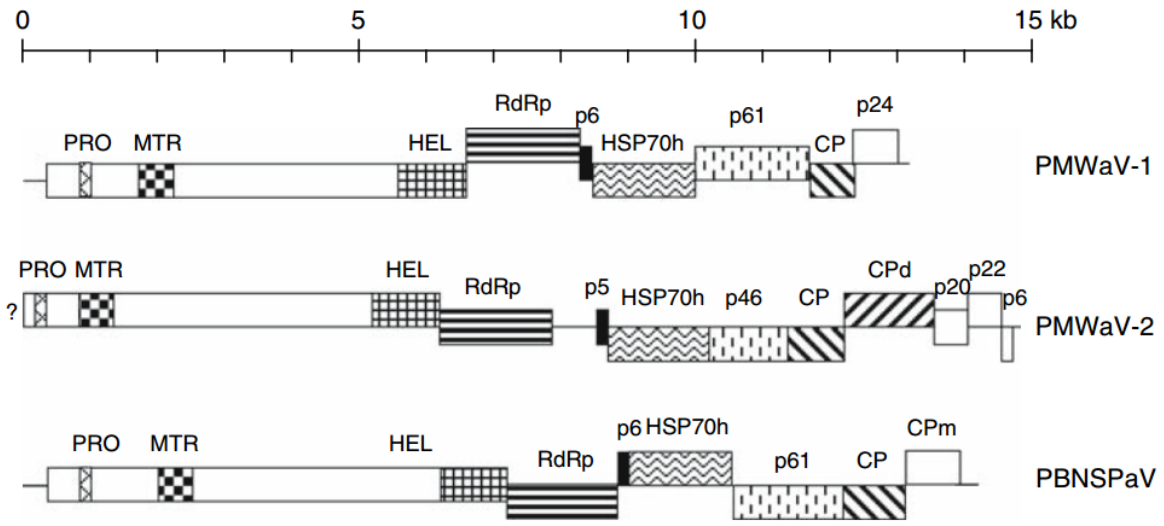

Figura 4 Comparação dos genomas de PMWaV-1, PMWaV-2 e PBNSPaV. Domínios: protease (PRO); metiltransferase (MTR); helicase (HEL); RNA polimerase dependente de RNA (RdRp); proteína homóloga a de choque térmico 70 (HSP70h); proteína capsidial (CP); proteína capsidial duplicada (CPd). Fonte: (MELZER et al., 2008).

Em 2009, o genoma de PMWaV-3 foi publicado. Com base na identidade dos genes ortólogos das ORFs de PMWaV-1 e PMWaV-2, foi proposto este vírus. Seu genoma estimado em 14Kb compreende 7 ORFs, não apresenta a região intergênica ORF 1b e ORF 2, e não codifica a proteína capsidial duplicada. A proteína da capa protéica codificada é estimada em 28,8 KDa (Figura 5) (SETHER et al., 2009).

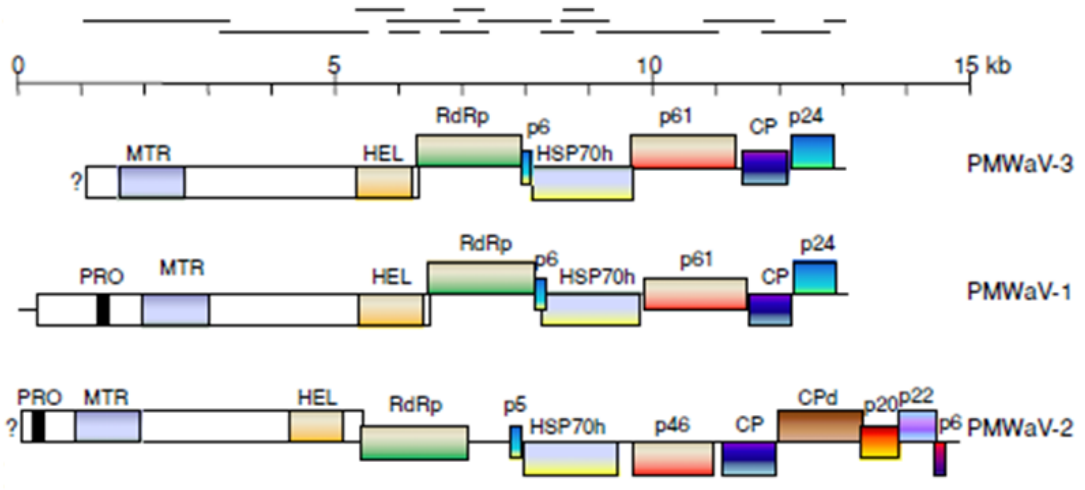

Figura 5 Comparação dos genomas dos três principais vírus do complexo PMWaV, o PMWaV-1, PMWaV-2 e PMWaV -3. Domínios: protease (PRO); metiltransferase (MTR); helicase (HEL); RNA polimerase dependente de RNA (RdRp); proteína homóloga de choque térmico 70 (HSP70h); proteína capsidial (CP); proteína capsidial duplicada (CPd). Fonte: SETHER, et al (2009).

Análises filogenéticas dos domínios e das ORFs dos membros da família *Closteroviridae* indicam a existência de dois clados distintos no gênero *Ampelovirus*. Dessa forma, PMWaV-1 e PMWaV-3 estariam agrupados com GLRaV-4 e PBNSPaV enquanto que PMWaV-2 estaria agrupado com GLRaV-3, -1 e LChV-2 (Figura 6) (SETHER et al., 2009).

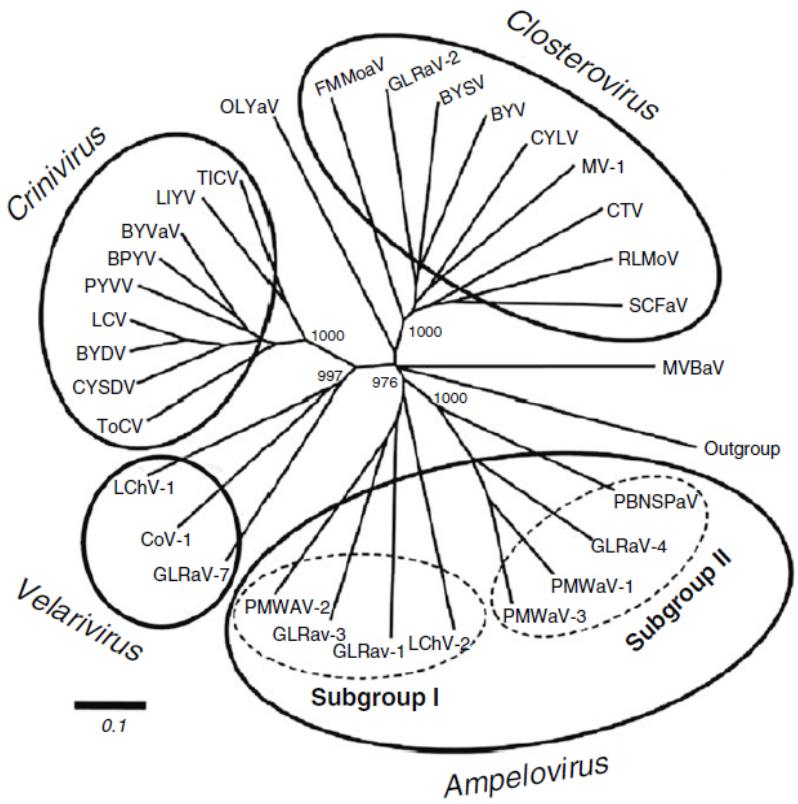

Figura 6 Árvore filogenética construída com base nas sequências de aminoácidos do gene HSP70h dos membros da família *Closteroviridae*. Observa-se no gênero *Ampelovírus*, dois subgrupos. No subgrupo I consta o vírus PMWaV-2 agrupado com GLRaV-3, GLRaV-1 e LChV-2. No subgrupo II constam os vírus PMWaV-1 e PMWaV-3 agrupados com GLRaV-4 e PBNSPaV. Fonte: (AGRANOVSKY, 2016).

3.2.3 Mecanismo de infecção dos Closterovirus

Vírus do gênero *Ampelovirus* são transmitidos às plantas de forma semi-persistente por cochonilhas. Quando na planta são encontrados exclusivamente em célculas do floema, característica da família *Closteroviridae* (SETHER; HU, 2002).

A replicação destes vírus é citoplasmática e ocorre a partir do momento em que penetram as células. Trata-se de um conjunto de vírus de RNA positivo [ssRNA(+)], portanto, servem de molde para a tradução das proteínas virais pelos ribossomos da célula hospedeira. Além disso, são encontrados na forma de dsRNA em vesículas que se acumulam no citoplasma das células, podendo surgir à partir da proliferação do retículo endoplasmático ou da fragmentação das mitocôndrias (AGRANOVSKY, 2016; VIRALZONE, 2018).

No processo de infecção, os closterovirus induzem na célula hospedeira a formação de um complexo multivesicular que aparenta ser o local de replicação. Inicialmente, na região 5’ a ORF é traduzida diretamente à partir do RNA genômico e codifica proteínas relacionadas com a replicação. A expressão do genoma ocorre por processamento proteolítico e tradução ribossomal +1 *frameshifting* para genes localizados na região proximal 5' enquanto na região 3'-terminal, os genes são expressos por meio de mRNA subgenômicos (AGRANOVSKY, 2016). Nesse processo, após a síntese da *RdRp* viral (ORF1b), os genes virais são transcritos em mRNA subgenômicos finalizados com a mesma sequência 3’. Concomitantemente, uma fita complementar a fita senso positiva é formada servindo de molde para a síntese do genoma viral (+)ssRNA. O deslocamento viral ocorre através de plasmodesmos de célula a célula e via floema para toda a planta (SETHER; HU, 2002).

Plantas infectadas por vírus respondem a infecção acionando mecanismos de defesa especialmente o silenciamento do RNA viral. O mecanismo destina-se a inviabilizar a tradução de proteínas virais ou ainda a degradar o RNA mensageiro. Em experimento com *N. benthamiana* foi avaliado o potencial de supressão do silenciamento local e sistêmico dos vírus PMWaV à partir de 7 ORFs (Hsp70, p46, CP, Cpd, p20, p22 e p6) da porção 3'-terminal de PMWaV-2 e 4 (Hsp70, p61, CP e p24) de PMWaV-1. Das ORFs avaliadas em PMWaV-2, 2 (P20 e CP) codificam proteínas com potencial de supressão tanto local como sistêmico e outras duas (P22 e CPd) codificam proteínas com ação sistêmica. Contudo, das ORFs avaliadas de PMWaV-1 apenas a que codifica p61 apresentou atividade de supressão do silenciamento sistêmico. Como os sintomas das doenças de origem viral ocorrem mediante a habilidade do patógeno em suprimir o mecanismo de silenciamento de RNA da planta hospedeira, a expressão de um complexo de proteínas supressoras por PMWaV-2 o torna eficientemente patogênico (DEY et al., 2015).

3.2.4 Mecanismos de resistência vegetal a patógenos

O reconhecimento do patógeno pela planta hospedeira requer a interação dos elicitores com receptores ancorados na membrana plasmática. Logo após, inicia-se uma cascata de sinalização desencadeando uma ampla gama de mecanismos de defesa, protegendo as plantas contra uma possível invasão do patógeno além de ativar uma reposta de hipersensibilidade (HR) para conter a infecção mediante morte celular (RESENDE et al., 2010).

Nesse processo, assim que ocorre o reconhecimento do elicitor, a primeira resposta de defesa é a explosão oxidativa, ou geração de espécies reativas de oxigênio (ROS). Assim, o peróxido de hidrogênio (H_2O_2) é produzido apresentando efeito direto sobre o patógeno e atuando no reforço da parede celular com a deposição de calose e o acúmulo de proteínas relacionadas à patogênese (PRs). Estas últimas incluem quitinases, glucanases e proteases, que afetam negativamente a colonização de patógenos (VAN LOON; GERAATS; LINTHORST, 2006). Essa resposta imediata à infecção corresponde a HR, anteriormente denominada de defesa basal, que confere certo nível de resistência a patógenos virulentos e culmina com a morte celular localizada (JOHAL; HULBERT; BRIGGS, 1995; VLOT; DEMPSEY; KLESSIG, 2009).

A cascata de sinalização do reconhecimento do *stress* envolve muitas moléculas em rotas metabólicas diferentes. Frequentemente associados à indução da expressão de genes de defesa, os fitohormônios ácido salicílico (AS), ácido abscísico (ABA), ácido jasmônico (AJ) e seu metil ester, metil jasmonato (MeJa) e o etileno (ET), podem atuar de forma sinérgica ou antagônica dependendo do elicitor (PERVIEUX et al., 2004). Além da sinalização hormonal, ROS atua como mensageiro secundário na modulação hormonal. É sabido também que a interligação de vias de sinalização interagem com a formação do AJ. Ainda não se tem esclarecido o mecanismo, contudo, a forma com que este hormônio é translocado pelo vegetal a grandes distâncias o coloca em posição de destaque como mediador da resistência local e sistêmica (PINTO; RIBEIRO; OLIVEIRA, 2011; STRATMANN, 2003).

O transporte dos sinais indutores da expressão de genes de resistência pelo floema para toda a planta promove a indução da via SAR (resistência sistêmica adquirida). Nesse contexto, AS, Auxina (TRUMAN et al., 2010), AJ (TRUMAN et al., 2007), ET (GLAZEBROOK, 2005), e ROS (WANG et al., 2014) ativam a via SAR em resposta a uma variedade de patógenos (GAO et al., 2015). Estudos tem demonstrado a presença de múltiplos sinais na ativação desta via podendo atuar de forma sinérgica ou antagônica, dependendo do patógeno. A integração destes sinais é discutida para o modelo AS-AJ/ET (MUR, 2005) e AJ-Auxina (TRUMAN et al., 2010). A importância da sinalização da auxina no estabelecimento de SAR pode resultar da modulação de AS-AJ/ET. Supõe-se que ocorra uma relação temporal e espacial da regulação desses hormônios ainda não elucidada (TRUMAN et al., 2010).

Além dos mecanismos de ampla resposta, HR e SAR, que atuam de forma local e sistêmica, as plantas possuem mecanismo de resistência específico ao patógeno como a degradação do genoma viral ou repressão da tradução pelo complexo RISC, ou ainda, a ubiquitinação de proteínas virais para a degradação via proteassoma. Em geral, na resposta antiviral, sRNAs são incorporados em um complexo de efetores chamado complexo de silenciamento induzido pelo RNA (RISC, do inglês *RNA-induced silencing complex*), que vai degradar o RNA viral (DING, 2010). As plantas também têm um mecanismo de amplificação que aumenta a resposta antiviral através do recrutamento de RNA polimerase dependente de RNA (RdRps) (CHITWOOD; TIMMERMANS, 2010). Esta amplificação secundária acredita-se ser essencial para o movimento do sinal de silenciamento sistêmico. Existem diversas matrizes de supressores do silenciamento, a exemplo, a proteína 2b do vírus do mosaico do pepino (CMV) que interage com ARGONAUTE1 (AGO1), do complexo RISC (ZHANG et al., 2006).

Quando ocorre a tradução das proteínas virais a planta ainda é capaz de silenciar o vírus através da via ubiquitina-proteassoma. Para que obtenha sucesso na degradação das proteínas virais, chaperonas são recrutadas para o reconhecimento e ligação da ubiquitina com a proteína alvo na interação com a enzima E3 (ubiquitina ligase). O complexo de ubiquitina ligase E3 constitui o SCF (SKP, CUL, F-box) que catalisa a ubiquitinação das proteínas destinadas à degradação via proteassoma. O reconhecimento da proteína alvo é mediado pelas proteínas do tipo F-box que

interligam os alvos à proteína SKP1. Tanto a Auxina quanto o AJ estimulam complexos SCFs que contenham seus respectivos receptores do tipo F-box. Assim, repressores AIA/IAA e o fator de transcrição JAZ, são degradados na presença de auxina e AJ respectivamente. Dessa forma, na presença desses hormônios, o complexo se torna ativo direcionando proteínas ubiquitinadas ao proteassoma 26S no citoplasma (QI et al., 2011). Esse é mais um mecanismo de defesa modulado pelo AJ e auxina.

Além disso, quando ocorre distúrbio na regulação da expressão protéica, a pressão de proteínas não dobradas sobre o retículo endoplasmático, ativa a via de resposta à proteína desdobrada UPR (do ingkês, unfolded protein response). Nesta via estão envolvidas as chaperonas BiP, calmodulina e calreticulina que atuam na modulação das proteínas liberadas pelo retículo. BiP apresenta um papel chave nesta regulação. Quando requerida em situações de estresse como o acúmulo de proteínas virais, proteínas dissociadas sinalizam respostas para estabilizar a homeostase aumento a expressão de mais chaperonas ou parando a tradução sinalizando para apoptose (HÜTTNER; STRASSER, 2012; ZHANG; WANG, 2012). BiP também é requerida para a expressão de proteínas de defesa. A regulação da expressão de BiP é condicionada por NPR1 (*Nonexpressor of pathogenesis-related proteins*), um regulador chave da via de SAR e responsivo a AS. O tratamento com benzotiadiazol-éster metílico (BTH), análogo AS, em mutantes de Arabidopsis *bip 2*, revelou a indução de morte celular como resposta ao acúmulo de proteína induzida por NPR1, além da redução na resistência a *Pseudomonas syringae* pv. *maculicola* ES4326. Esta relação foi confirmada quando o mesmo tratamento foi aplicado ao duplo mutante *bip2* e *npr1* e nenhuma morte celular foi observada. Assim, foi demonstrado o efeito do tratamento por AS sobre as defesas da planta na ausência do perfeito equilíbrio da expressão de *BiP* podendo levar a suscetibilidade ao patógeno (WANG, 2005).

Por fim, as plantas codificam proteínas de resistência específica a determinado patógeno através da expressão dos genes R (do inglês *R genes*). Estes genes são ativados na percepção dos elicitores. Nesse caso, a interação depende da presença na planta do gene R de herança dominante que reconhece o patógeno com seu gene dominante de avirulência *Avr*. Não havendo compatibilidade, a doença se

instala (PINTO; RIBEIRO; OLIVEIRA, 2011). Quando ocorre compatibilidade, as proteínas são traduzidas. A principal classe dessas proteínas apresenta o domínio de ligação aos nucleotídeos, rico em leucina e são conhecidas por NBS-LRR (MCHALE et al., 2006). Embora não se conheça completamente o mecanismo, em *Arabidopsis* foi demonstrada a função de monitoração das proteínas alvos vegetais permitindo o acionamento de um pequeno número de proteínas NBS-LRR para a detecção de um grande número de efetores patogênicos. Dessa forma, a vulnerabilidade da planta é reduzida. Nesse estudo, proteínas CNL detectaram a fosforilação de RPM1 pelo efetor AvrB de *Pseudomonas syrungase* pv. *glycinea* sendo induzida a resposta de resistência. Muitas enzimas incluindo do tipo NBS-LRR necessitam de metais na sua composição para diversos processos biológicos e podem ser induzidas por AJ (REYMOND, 2000) e ET (SIVASANKAR; SHELDRICK; ROTHSTEIN, 2000). Outro grupo de genes R, conhecidos por genes *RTM* (Restricted Tobacco etch virus Movement), codificam proteínas que interagem com proteínas de movimento do virus TEV restringindo o seu movimento via floema em *Arabidospsis* sem ativar HR ou SAR (CHISHOLM et al., 2001; WHITHAM et al., 2000; YAMAJI et al., 2012).

Assim, na interação planta-patógeno estão envolvidas muitas vias metabólicas moduladas por hormônios que atuam de forma orquestrada para determinar o nível de resistência ou tolerância da planta. A integração dos mecanismos que determinam a resistência é dependente do tipo de patógeno. A compreensão dos principais elementos chaves nesta maquinaria pode determinar estudos que visam o melhoramento dos vegetais com foco no patógeno de interesse.

3.3 MANEJO DA MURCHA DO ABACAXIZEIRO

As medidas de contenção da doença implicam em restringir o acesso das cochonilhas às plantas de abacaxi e dessa forma intervir na transmissibilidade dos vírus e na indução de sintomas. Sendo assim, o manejo da doença compreende o uso de inseticidas que matam as cochonilhas, e na eliminação das plantas identificadas como foco propagativo da doença. O uso indiscriminado de substâncias químicas no campo elimina não apenas o inseto vetor, mas também seus inimigos

naturais desestabilizando o ecossistema além de viabilizar a degradação do meio ambiente.

Tais medidas não são suficientes para conter a dispersão dos vírus uma vez que os sintomas demoram a aparecer o que oportuniza o uso de uma planta infectada como planta mãe para obtenção de mudas. Uma vez estas mudas no campo, sem controle fitossanitário, os sintomas poderão ser induzidos podendo as plantas sequer florir ou frutificar. Além disso, podem ainda servir como fonte de inoculo primário na nova plantação contribuindo para a dispersão dos vírus a longas distâncias (MELZER et al., 2001; SETHER et al., 2005).

A dificuldade em conter MWP aponta para a necessidade em certificar a ausência dos vírus PMWaV nas mudas, podendo ser adotados o uso de cultura de tecidos ou ainda futuros cultivares resistentes ao PMWaV. Neste sentido, experimentos de tratamento térmico de coroas de abacaxi alcançaram êxito eliminando o vírus PMWaV-1 (SETHER et al., 2001). Recentemente, o tratamento hidrotérmico de mudas do tipo filhote da cultivar Pérola (56º C por 30 min) revelou resultados promissores para a eliminação do PMWaV-2 não tendo sido detectado via RT-PCR nas novas raízes após o tratamento (LOPES, 2018).

Como estratégia para o desenvolvimento de uma cultivar resistente ao vírus mais relatado como agente causal da MWP, o PMWaV-2, foram construídos abacaxizeiros transgênicos. A transformação de brotos micropropagados e de secções de caule foi mediada por *Agrobacterium tumefaciens* contento o construto. Neste construto, o gene que codifica a proteína de revestimento do vírus, a CP de PMWaV-2, foi inserida com repetição invertida. Os abacaxizeiros certificados como transgênicos foram avaliados em ensaios conduzidos em casa de vegetação. Os testes demonstraram que cerca de 10% das plantas foram resistentes ao PMWaV-2 (PEREZ et al., 2006).

O cultivo de plantas resistentes ou tolerantes ao PMWaV contribui para o aumento da produtividade do abacaxizeiro e para a preservação do meio ambiente. Além de reduzir o uso de pesticidas economizando com insumos e mão-de-obra, tal prática

também reduz a infiltração de produtos químicos no solo e evita desequilíbrio no ecossistema.

A obtenção do cultivar geneticamente aprimorado requer o estudo do transcriptoma de plantas sintomáticas e assintomáticas infectadas por PMWaV, especialmente o PMWaV-2. Essa estratégia pode direcionar o melhoramento genético de cultivares apontando vias metabólicas alvos de modulação durante a suscetibilidade ou resistência a infecção viral.

3.4 USO DO RNAseq NO ESTUDO DA INTERAÇÃO PLANTA-PATÓGENO

As tecnologias do sequenciamento de nova geração (NGS, do inglês *Next-generation sequencing*), fornecem uma abordagem ampla para estudos do perfil de expressão gênica em comparação as técnicas anteriormente utilizadas como microarranjos e análise serial de expressão gênica (SAGE). Muitas questões biológicas vem sendo respondidas com base na revelação do mecanismo de regulação transcripcional desvendado pelo uso de NGS (JAIN, 2012).

O sequenciamento do RNA (RNAseq) permite identificar todos os RNAs expressos em dado momento, célula ou tecido, mantendo o foco da pesquisa nas porções gênicas do genoma. Além disso, permite comparar o perfil de expressão gênica em diferentes condições ambientais, estados patológicos, fisiológicos ou de desenvolvimento. Também permite caracterizar alternativas de *splicing* e polimorfismo de um nucleotídeo (SNPs). Neste sentido, diversas plataformas de sequenciamento e softwares de bioinformática foram e continuam sendo gerados para otimizar estudos de expressão gênica (JAIN, 2012; MARTIN; WANG, 2011; MOROZOVA; HIRST; MARRA, 2009).

Assim, o RNAseq é bastante promissor tendo sido utilizado para avaliar a interação entre plantas e seus patógenos como bactérias, fungos e vírus em condições de casa de vegetação ou sob condições de campo. A exemplo, para compreender a patogênese ferrugem amarela em trigo infectado pelo fungo *Puccinia striiformis* f. sp. *tritici*, a análise do transcriptoma à partir de folhas por RNAseq, montagem *de novo* e

ferramentas computacionais interpretativas permitiu propor genes candidatos envolvidos na resistência da planta. Nesse experimento foram avaliadas plantas inoculadas e não inoculadas como controle no ensaio conduzido em casa de vegetação (HAO et al., 2016). Da mesma forma, a resistência de plantas *Brachypodium distachyon* à inoculação do vírus *Barley stripe mosaic vírus,* e cultivadas em casa de vegetação, foi avaliada por RNAseq com mapeamento das sequências obtidas contra o genoma de referência sendo possível contribuir para o entendimento dos mecanismos moleculares de resistência (WANG et al., 2017).

O estudo do sistema de interação planta-vírus também tem sido avaliado à partir de plantas infectadas sintomáticas e assintomáticas em seu habitat natural. Recentemente, em plantas de *Arabidopsis* foram avaliados os transcriptomas de vários vírus de forma simultânea através do RNAseq. Com o uso de ferramentas de bioinformática foi possível obter informações relacionadas tanto aos vírus presentes no conjunto de plantas como a identificação dos genes diferencialmente expressos em razão da infecção. Além disso, os genes foram identificados com base no genoma de referência enquanto os genomas virais foram identificados por meio da montagem *de novo* (KAMITANI et al., 2016).

Diante do exposto, o uso do RNAseq aliado às ferramentas de bioinformática constituem a alternativa ideal para o estudo de expressão gênica com fins de melhoramento vegetal. O transcriptoma do abacaxi vem sendo estudado para fins de melhoramento da produção. Pesquisas usando RNAseq e montagem *de novo* já foram conduzidas para a compreensão do amadurecimento do fruto de abacaxi (LIU; FAN, 2016; ONG; VOO; KUMAR, 2012) e para a resistência da planta ao frio (CHEN et al., 2016). Contudo, este estudo é pioneiro em pesquisas do transcriptoma de *A. comosus* que objetiva avaliar a interação abacaxi-PMWaV cultivados no habitat natural usando o genoma recentemente publicado como referência.

4 ESTRATÉGIA DE AÇÃO

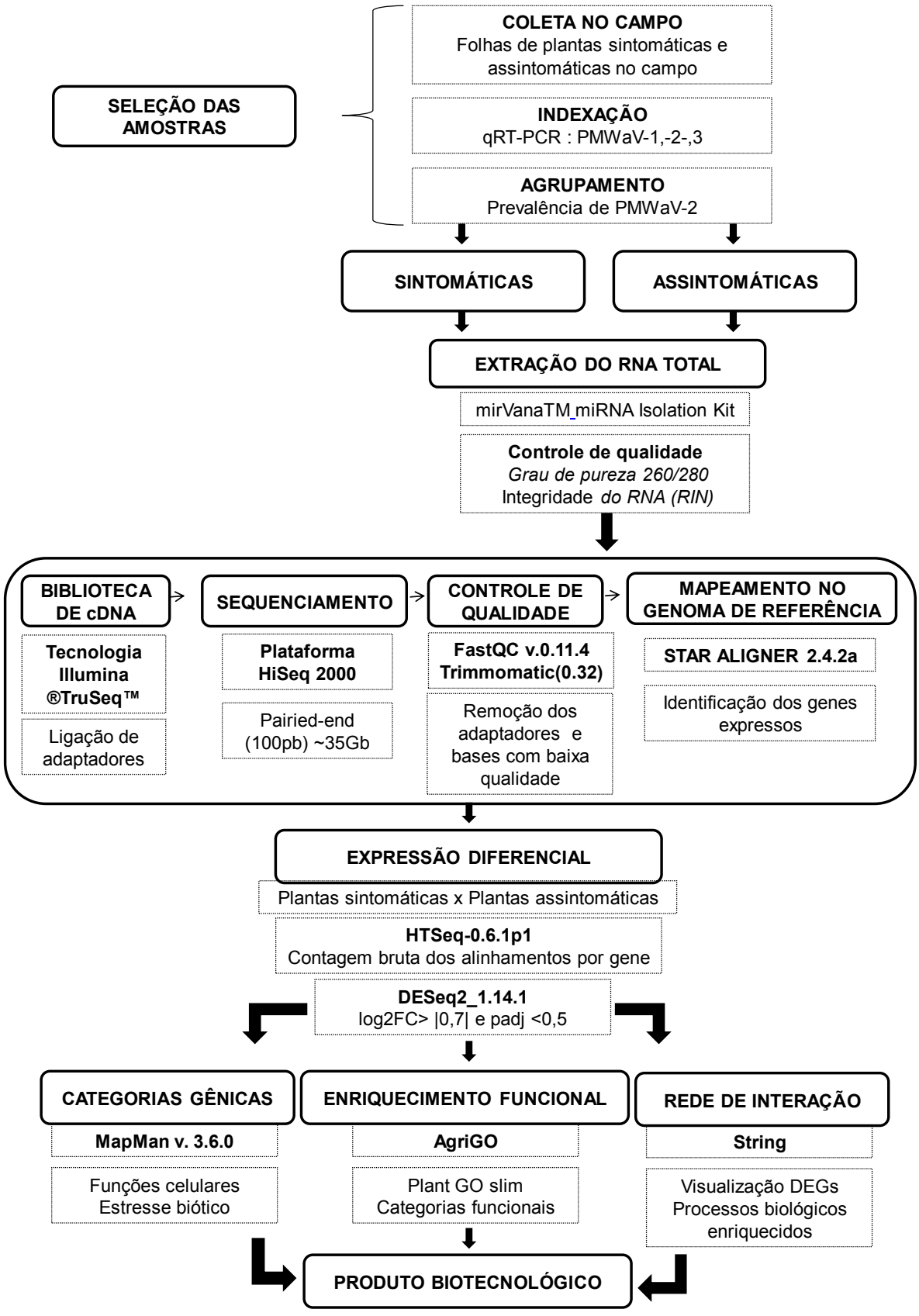

Figura 7 Fluxograma das atividades estretégicas para o atendimento aos objetivos desta Tese.

5 CAPÍTULOS

5.1 CAPÍTULO 1

- Artigo submetido e aceito para publicação na Revista Acta Horticulturae em 30 de março de 2018.

Bioinformatics approach to the study of the molecular behavior of mealybug wilt of pineapple

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Abstract

Mealybug wilt of pineapple (MWP) is a disease caused by the *Pineapple mealybug wilt-associated virus* (PMWaV) complex transmitted by *Dysmicoccus brevipes* and *D. neobrevipes*. MWP symptoms are characterized by root dessication, leaf wilting and consequent failure to produce a fruit. The molecular mechanisms involved in the pineapple-PMWaV interaction for MWP symptomatology are still unclear. In this work, messenger RNAs of asymptomatic and symptomatic pineapple plants were evaluated using Illumina RNA sequencing technology. From a total of 79 million reads per sample, 16,097 genes were identified using STAR aligner and HTseq for paired-end files. Differentially expressed genes (DEGs) between the evaluated groups were estimated using DESeq2 and edgeR, with an FDR cutoff of ≤ 0.05. A total of 207 DEGs were detected, with 61 up-regulated and 146 down-regulated in symptomatic plants infected by PMWaV-2. The methodologies improved by the assays presented in this article and the detected DEGs can substantiate further researches with pineapple and the MWP disease.

Keywords: Bioinformatics, pineapple, transcriptomic, PMWaV, STAR, DESeq2, edgeR.

INTRODUCTION

Mealybug wilt of pineapple (MWP) can lead to losses of up to 100% of production, affecting producers in Hawaii (Sether and Hu, 2001; Sether and Hu, 2002a), Brazil (Ventura and Zambolim 2002), Cuba (Borroto-Fernandez et al.2007), Australia (Gambley et al. 2008) and Taiwan (Shen et al., 2009).

Severe symptoms of MWP begin with root dessication followed by wilting and gradual discoloration (intense reddening) of leaves, which curl towards the soil and become completely dry at the tips. Infected plants may fail to fruit or produce an atrophied and withered that is unsuitable for use fresh or for processing (Ventura and Zambolim 2002). MWP is caused by a virus complex, the *Pineapple mealybug wilt-associated virus* (PMWaV), transmitted by the mealybugs *Dysmicoccus brevipes* and *D. neobrevipes* (Sether, Ullman, and Hu 1998; Sether and Hu, 2002b).

PMWaV-1, PMWaV-2, and PMWaV-3 are associated with MWP disease development in pineapple. PMWaV-2 has been consistently reported in producer countries, except in Australia, as the main virus that causes MWP symptoms. Other PMWaVs are associated with reduced production but not with severe disease symptoms (Borroto-Fernandez et al. 2007; Gambley et al. 2008; Sether et al. 2005; Shen et al. 2009). The studies carried out to understand the etiology of MWP focused on determining the causal agent, its molecular interaction with the plant and

improvement of the plant based on the study of the pathogen genome and clonal cleansing (Melzer et al. 2001; Perez et al. 2006; Sether et al. 2001). In addition, the study of pathogenicity factors of PMWaV-1 and PMWaV-2 in *Nicotiana benthamiana* (Dey et al., 2015), revealed the potential suppressive action of RNA silencing of plant contributing to the understanding of pineapple-PMWaV. However, the molecular mechanisms involved in the host plant response to PMWaV is not yet known.

In studies of the set of molecules produced by the plant in response to the pathogen, at the transcriptional level it is possible to evaluate the set of messenger RNAs (mRNA) and to determine the activity of the genes (Adams, 2008; Sinha and Smith, 2014; Hao et al., 2015; Kamitani et al., 2016). With the advent of a new generation of sequencing technologies (NGS), traditional methodologies such as microarray, serial gene expression analysis (SAGE), and Sanger sequencing were complemented by deep RNA sequencing **(**RNAseq) (Jain, 2012). In this context, RNAseq allows the identification of all RNAs expressed in cells or tissue at any given time. In addition, the comparison of the gene expression profile in different environmental conditions or pathological, physiological or developmental states can be performed. Also, with this technique, it is possible to characterize alternative splicing and polymorphism of one nucleotide (SNPs). In this sense, several sequencing platforms and bioinformatics softwares have been generated to optimize studies of gene expression (Morozova et al., 2009; Martin and Wang, 2011; Jain, 2012).

Many factors influence the differential expression studies through RNA-seq. Aspects such as depth of sequencing, coverage, material quality, biological variability, and the statistical model adopted to evaluate the abundance of the transcripts can generate false positive results and compromise the validation and interpretation of the results. Therefore, making careful choices, not only with the experimental design but also with the bioinformatic tools used, is essential for the estimation of the differentially expressed genes (DEGs). In this context, for comparative analysis of small numbers of samples, DESeq and edgeR are widely used tools with similar methodology but with differences in the normalization of the data and in the estimative of dispersion of the genes in the sample and in the group (Anders et al., 2013).

Based on the *Ananas comosus* genome (Ming et al., 2015), the transcriptome of asymptomatic and symptomatic 'Smooth Cayenne' pineapple plants was sequenced and evaluated to locate differentially expressed genes with the objective of identifying the differences in the gene expression involved in the pineapple-PMWaV-2 interaction.

MATERIALS AND METHODS

Plant materials and symptom conditions

Pineapple plants (*A.comosus* var *comosus*) 'Smooth Cayenne', were cultivated in an experimental field at the farm of the Capixaba Institute for Research, Technical Assistance and Rural Extension (INCAPER, Sooretama, ES, Brazil). Source material consisted of, three vegetative plants with severe symptoms of MWP designated as symptomatic (Figure 1B). Likewise, three asymptomatic plants formed a second group (Figure 1A). For viral indexing, and transcriptome analysis, D leaves were removed, quickly washed with distilled water and ethanol (70%), and immediately frozen in an ethanol bath with dry ice, then transported from the field in dry ice.

Figure 1. Asymptomatic (A) and symptomatic (B) pineapple plants.

Total RNA extraction, cDNA, and qPCR

Total RNA was extracted from the basal portion of the leaves (~ 100 mg) using the mirVana™ miRNA Isolation Kit (Life Technologies, Carlsbad, CA) and the RNeasy® Plant Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's protocol. The quality of total RNA was measured by the A260/280 and A260/230 ratios in a Nanodrop 2000 spectrophotometer apparatus (Thermo Scientific, Wilmington, USA). Total RNA aliquots of ratio 2.0 and a with high yield of total RNA were sent to sequencing (Table 1). From 10 ng of the total RNA extract, reverse-transcribed cDNA was obtained using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Cheshire, UK).

Diagnosis of the relative expression of PMWaV-1, PMWaV-2, and PMWaV-3 was conducted using qPCR 7500 version 2.0.1 (Applied Biosystems) according to the protocol of the Biotechnology Laboratory (UFES/BioTec). The cDNA was used as a template for real-time PCR with 10 µl of PCR Master Mix (Applied Biosystems) and virus-specific primers in a reaction of 20 µl. This mixture was also used for detection of actin as an endogenous control. Specific sense and antisense primers PMWaV-1-F (5'-GCAGGCGGTAGTAAACGAA-3') and PMWaV-1-R (5'-AAGTGCCTCCTCCGAAATC-3') for detection of PMWaV-1, PMWaV-2-F (5'-ACGGTACCAGCCGACTACA-3') and PMWaV-2-R (5'-CAGCGGTCGGTTCATTTAC-3') for detection of PMWaV-2, and PMWaV-3-F (5'-TGACGTTGTCGGTGTGTTC-3') and PMWaV-3-R (5'-ACCACCGCCTGTACGTTTA-3') for detection of PMWaV-3 were used, and for detection of actin actin-F (5'-CGTTTGCGACAATGGAACTG-3') and actin-R (5'- CGCTCTCGGTGCATCATCT-3') primers were used. The reaction mixture was heated at 95 °C for 10 min; amplification occurred for 40 cycles of 15 s at 95 °C followed by 1 min at 60 °C. A reaction mixture without addition of cDNA was used as a negative control. Relative expression was estimated by the $2^{-\Delta\Delta Ct}$ method, and the difference was evaluated by the t-test with standard error calculations between the groups of pineapple plants.

Sequencing, quality control, and filtering

Biological triplicates were selected for deep sequencing. Aliquots of more than 4 µg of total RNA from each sample were sent to Macrogen Korea, Seoul, Republic of Korea, where RNA integrity was confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). The integrity of the RNA was determined by the ratio of 28S:18S ribosomal RNA. This measure is estimated by automated microcapillary electrophoresis associated with the algorithm that determines RNA integrity (RIN). This number, ranging from 1 to 10, determines the level of RNA degradation from the most degraded to the intact (Schroeder, et al. 2006). Aliquots of total RNA with RIN between 6.9 and 8.2 were used (Table 1). Paired-end libraries with fragments above 278 bp were prepared using Illumina's TruSeq technology, and sequencing was conducted on a flow cell of the HiSeq 2000 platform (Illumina, San Diego, CA) generating paired-end reads of 101bp. The quality of the obtained reads was verified by FastQC v.0.11.4 (Andrews 2010) and treated using Trimmomatic (0.32) (Bolger et al. 2014) to remove low-quality reads.

Mapping of reads to the pineapple genome and differential expression profile

Treated reads were aligned to the *Ananas comosus* v3 reference genome (Ming et al. 2015) obtained from the JGI Phytozome v.11.0.9 database (http://phytozome.jgi.doe.gov/). The alignment of the reads was performed using STAR_2.4.2a (Dobin et al. 2013), distributed under the GPLv3 license, using the default parameters suggested by the developers. The counts of the reads mapped to each gene per sample was performed using HTSeq-0.6.1p1 (Anders et al. 2015). DEGs were estimated in duplicates using the packages DESeq2_1.14.1 (Love et al. 2014) and edgeR_3.18.1 (Robinson et al. 2010). A gene was considered differentially expressed if it presented a adjusted p-value < 0.05 for DESeq2 and FDR < 0.05 for edgeR.

RESULTS AND DISCUSSION

PMWaV indexation in symptomatic and asymptomatic pineapple plants

Estimates of expression of the PMWaV-1 and PMWaV-3 were similar in symptomatic and asymptomatic plants whereas that PMWaV-2 was estimated in symptomatic pineapple plants with a significant difference ($p = 0.02$) (Figure 2).

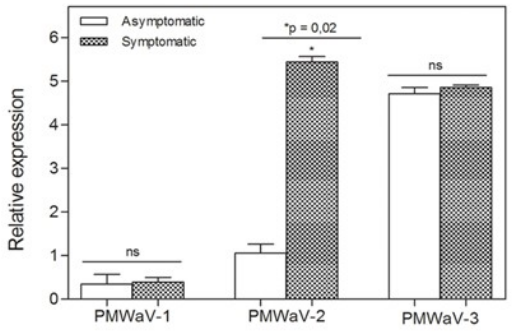

Figure 2. Relative expression profile of PMWaV-1, PMWaV-2, and PMWaV-3 in asymptomatic and symptomatic pineapple plants.

The absence of MWP symptoms in PMWaV-3-infected pineapple plants has been reported in Hawaii (Sether and Hu 2001), Taiwan (Shen et al. 2009) and Brazil (Peron et al. 2009). Similarly, PMWaV-1 was characterized as a virus that has only one pathogenicity factor with systemic suppression activity of the RNA-silencing mechanism and not directly involved in the severity of the MWP disease and, frequently detected in symptomatic pineapple plants only in the presence of PMWaV-2 (Dey et al. 2015). In contrast, the PMWaV-2 was detected in symptomatic plants worldwide as the aetiological agent of MWP except in Australia (Sether and Hu 2002b; Sether et al. 2005; Shen et al. 2009; Peron et al. 2009). Suppression factors of local and systemic RNA silencing in host plants were suggested to be potential determinants in the infection of PMWaV-2 (Dey et al. 2015).

Sample Screening

The RNA samples extracted with the two RNA extraction kits showed a good yield in micrograms (μg) and purity quality (260/280 and 260/230). However, although the amount of RNA produced in both kits exceeded the amount required by the sequencing platform, only the mirVana ™ miRNA Isolation Kit resulted in samples with an acceptable minimum RIN value (~7) for analysis (Table 1).

Table 1. The degree of purity analysis and total RNA integrity (RIN).

Plant Sample	mirVana™ miRNA Isolation Kit				RNeasy® Plant Mini Kit			
	RNA (µg)	260/280	260/230	RIN	RNA (µg)	260/280	260/230	RIN
1 Asymptomatic	7.7	2.09	2.03	6.9	7.7	2.11	2.07	5.7
2 Asymptomatic	9.7	2.11	2.20	8.2	5.5	2.11	2.02	5.7
3 Asymptomatic	13.2	2.10	1.52	8.2	5.6	2.02	1.06	5.6
4 Symptomatic	8.5	2.10	2.22	7.7	7.3	2.06	0.89	6.2
5 Symptomatic	4.7	2.09	1.77	6.9	6.0	2.08	1.77	5.0
6 Symptomatic	5.7	2.07	1.41	7.5	9.8	2.05	1.75	4.2

The integrity of the RNA is fundamental so that the reads obtained will be effective in reconstructing the transcriptome, allowing the identification of the expressed genes. Thus, Figure 3 presents the result of RNA integrity analysis of sample 1 extracted with the two methods to illustrate the difference in the quality of the extracts obtained. Although the concentration of the total RNA in the two samples is the same, the definition and height of the rRNAs peaks reports the distribution of the total RNA degraded in B that occurs in a smaller amount in A. In general, the two kits can be used to recover total RNA with good quality and yield, but the mirVana™ miRNA Isolation Kit enabled the best set of aliquots for the RNAseq.

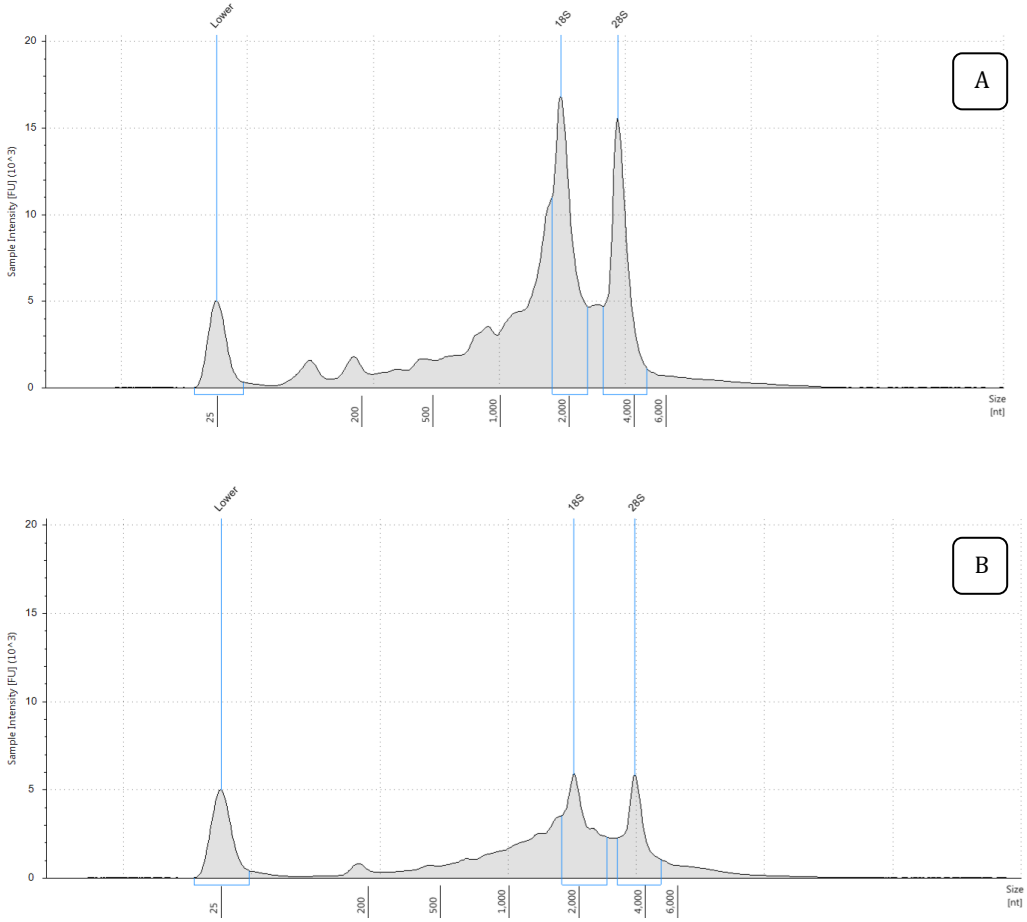

Figure 3. Measurement of the integrity of total RNA extracted with mirVana™ miRNA Isolation Kit (A) and RNeasy® Plant Mini Kit (B). (A)There are two well-defined peaks corresponding to the 18S and 28S ribosomal subunits and the ratio between the 28S and 18S peaks is approximately 2:1 whereas (B) is an example of partially degraded RNA.

Bioinformatics analysis

Sequencing of approximately 8.6 Gb per RNA sample generated an average of 85.2 Mb of reads with GC percentage higher than 47% and QC above 90% post trimming the low-quality bases (Table 2).

Table 2. Quality control (QC) of sequencing data after trimming.

Plant Sample	Total Bases	Read Count	GC (%)	Q20 (%)	Q30 (%)
1 Asymptomatic	7,763,048,084	81,522,142	47.12	98.25	90.81
2 Asymptomatic	8,482,766,697	89,312,412	47.63	98.17	90.48
3 Asymptomatic	6,745,197,116	71,111,876	48.19	98.15	90.39
4 Symptomatic	8,338,317,871	87,667,964	48.07	98.13	90.29
5 Symptomatic	6,857,534,339	71,862,930	47.64	98.18	90.5
6 Symptomatic	7,443,485,003	78,076,522	47.84	98.18	90.51

Approximately 80% of all trimmed reads were aligned to the reference genome, which allowed the detection of 16,097 genes. A total of 390 DEGs were detected by DESeq2 while only 223 were detected by edgeR (Figure 4).

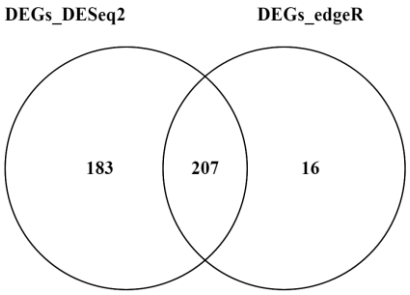

Figure 4. Graphical representation of the relationship between the lists of DEGs obtained by DESeq2 and edgeR packages.

DESeq2 and edgeR are packages recommended for robustness in determining the set of DEGs (Love et al. 2014; Zhou et al. 2014). However, DESeq2 computes the mean-variance relation in the data set while edgeR assumes a common dispersion to all genes. The count of reads per gene is determined by the total depth of genes per individual (Love et al. 2014). Moreover, DESeq2 allows the detection of genes with low expression but with a significant change between control and treatment groups. In addition, besides the characteristics of the software, the set DEGs_DESeq2 groups 93% of the genes detected by edgeR (Figure 4).

The intersection of the DEGs detected by DESeq2 and edgeR groups 146 down-regulated and 61 up-regulated in symptomatic plants. To direct the biological interpretation of the data obtained here, we believe that these 207 carefully selected genes can contribute to downstream research.

CONCLUSIONS

- Symptomatic plants showed PMWaV-2 as being an important source for the study of the mechanisms of interaction of the pineapple with the main virus that causes MWP.
- mirVana™ miRNA Isolation Kit appeared to be the best option to obtain candidate samples for RNAseq. In addition to the yield, the RNA quality was high, which confirms the efficiency of the method.
- The STAR was effective in mapping 16,097 genes and 80% of all reads were successfully aligned to the pineapple genome.
-Using DESeq2 and edgeR, 207 differentially expressed genes were detected in symptomatic plants, considering asymptomatic plants as control.
- This is the first study of the PMWaV-infected pineapple transcriptome and the data obtained will contribute to better understand the Mealybug wilt of pineapple disease.

ACKNOWLEDGEMENTS

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Literature Cited

Adams, J. (2008). Transcriptome: connecting the genome to gene function. Nature Education. *1* (1), 195.

Anders, S., McCarthy, D.J., Chen, Y., Okoniewski, M., Smyth, G.K., Huber, W. And Robinson, M.D. (2013) Count-based differential expression analysis of RNA sequencinf data using R and Bioconductor. Nature Protocols. *8*, p.1765-1786 http://doi.org/ 10.1038/nprot.2013.099.

Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq-A Python framework to work with high-throughput sequencing data. Bioinformatics. *31* (*2*), 166-169 http://dx.doi.org/10.1093/bioinformatics/btu638.

Andrews S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects.

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics, *30* (15), 2114–2120. http://doi.org/10.1093/bioinformatics/btu170.

Borroto-Fernandez, J.A., Costa T.A., and Laimer, M. (2007). RT-PCR detection and protein-protein interaction of viral components of Pineapple mealybug wilt-associated virus 2 in Cuba. Journal of Plant Pathology. *89,* 435–439 http://dx.doi.org/10.4454/jpp.v89i3.779.

Dey, K.K., Borth, W.B., Melzer, M.J., Wang, M.L, and Hu, J.S. (2015). Analysis of pineapple mealybug wilt associated virus -1 and -2 for potential RNA silencing suppressors and pathogenicity factors. Viruses *7* (*3*), 969–995 http://dx.doi.org/10.3390/v7030969.

Dobin, A.., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T. R. (2013). STAR: ultrafast universal RNA-Seq aligner. *29* (*1*), 15-21 Bioinformatics. http://dx.doi.org/10.1093/bioinformatics/bts635.

Gambley, C.F., Steele, V., Geering, A.D.W., and Thomas, J.E. (2008). The genetic diversity of ampeloviruses in Australian pineapples and their association with mealybug wilt disease. Australasian Plant Pathology *37* (*2*), 95–105. http://dx.doi.org/10.1071/AP07096.

Hao, Y., Wang, T., Wang, K., Wang, X., Fu, Y., Huang, L. and Kang, Z.(2016) Transcriptome Analysis Provides Insights into the Mechanisms Underlying Wheat Plant Resistance to Stripe Rust at the Adult Plant Stage. PLoS ONE. 11(*3*):e015071.2015 http://doi.org/10.1371/journal.pone.0150717.

Jain, M. (2012). Next-generation sequencing techonologies for gene expression profiling in plants. Briefings in functional genomics. 11 (*1*) p.63-70 http://doi.org/ 10.1093/bfgp/elr038.

Kamitani, M., Nagano, A.J., Honjo, M. N., Kudoh, H. (2016) RNA-Seq reveals virus-virus and virus-plant interactions in nature. FEMS Microbiol Ecol. 92(11) pii: fiw176 http://doi.org/10.1093/femsec/fiw176.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. Genome Biology. *15* (12), 550 http://dx.doi.org/10.1186/s13059-014-0550-8.

Martin, J.A. and Wang, Z. (2011). Next-generation transcriptome assembly. Nature Reviews Genetics.12 (*10*) p.671-682 http://doi.org/ 10.1038/nrg3068.

Melzer, M.J., Karasev, A.V., Sether, D.M., and Hu, J.S. (2001). Nucleotide sequence, genome organization and phylogenetic analysis of pineapple mealybug wilt-associated virus-2. Journal of General Virology. *82* (P*t 1*), 1-7 http://dx.doi.org/10.1099/0022-1317-82-1-1.

Ming, R., VanBuren, R., Wai, C.M., Tang, H., Schatz, M.C., Bowers, J.E., Lyons, E., et al. (2015). The pineapple genome and the evolution of CAM photosynthesis. Nature Genetics *47* (*12*), 1435–1442. http://dx.doi.org/10.1038/ng.3435.

Morozova, O., Hirst, M. and Marra, M. (2009). Applications of New Sequencing Technologies for Transcriptome Analysis. Annual Review Genomics Human Genetics.10 p.135-151http://doi.org/ 10.1146/annurev-genom-082908-145957.

Perez, P.E, Sether, D.M., Melzer, M.J., Busto, J.L., Nagai, C and Hu, J.S. (2006). Characterization and Control of Pineapple Mealybug Wilt Associated Ampeloviruses. ISHS Acta Horticukturae *702*, 23-27. http://doi.org/ 10.17660/ActaHortic.2006.702.1.

Peron, F N, P M B Fernandes, J A Ventura. (2009). Detecção de PMWaV-1 e PMWaV-2 em abacaxizeiros no Estado do Espírito Santo. Tropical Plant Pathology. *34,* S268-S268.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics, *26,* (*1*), 139-140 http://dx.doi.org/ 10.1093/bioinformatics/btp616.

Schroeder, A., Mueller, O., Stocker, S., Salowsky, R., Leiber, M., Gassmann, M., Lightfoot, S., Menzel, W., Granzow, M. And Ragg, T. (2006). The RIN: an RNA integrity values number for assigning integrity values to RNA measurements. BMC Molecular Biology. 7,3 https://doi.org/10.1186/1471-2199-7-3.

Sether, D.M, and Hu, J.S. (2002)a. Yield Impact and Spread of Pineapple Mealybug Wilt Associated Virus -2 and Mealybug Wilt of Pineapple in Hawaii. Plant Disease. *86* (*8*), 867–874. http://doi.org/10.1094/PDIS.2002.86.8.867.

Sether, D.M, and Hu, J.S. (2002)b. Closterovirus infection and mealybug exposure are necessary for the development of mealybug wilt of pineapple disease. Phytopathology. *92* (*9*), 928–935 http://doi.org/10.1094/PHYTO.2002.92.9.928.

Sether, D.M., and Hu, J.S. (2001). The impact of Pineapple mealybug wilt-associated virus-1 and reduced irrigation on pineapple yield. Australasian Plant Pathology. *30* (*1*), 31–36 http://dx.doi.org/10.1071/AP00060.

Sether, D.M., Melzer, M.J., Busto, J., Zee, F., and Hu, J.S. (2005). Diversity and Mealybug Transmissibility of Ampeloviruses in Pineapple. Plant Disease *89* (*5*), 450–456. http://doi.org/10.1094/pd-89-0450.

Sether, D.M., Ullman, D.E., and Hu, J. S. (1998). Transmission of Pineapple Mealybug Wilt-Associated Virus by Two Species of Mealybug (*Dysmicoccus* Spp.). Phytopathology. *88* (*11*), 1224-1230 http://doi.org/10.1094/PHYTO.1998.88.11.1224.

Sether, D.M., Karasev, A.V., Okumura, C., Arakawa, C., Zee, F., Kislan, M.M., Busto, J.L., and Hu, J.S. (2001). Differentiation, Distribution, and Elimination of Two Different Pineapple Mealybug Wilt-Associated Viruses Found in Pineapple. Plant Disease. *85* (*8*), 856–864 http://doi.org/10.1094/PDIS.2001.85.8.856.

Shen, B.N., Zheng, Y.X., Chen, W.H., Chang, T.Y., Ku, H.M., and Jan, F.J. (2009). Ocurrence and Molecular Characterization of Three Pineapple Mealybug Wilt-Associated Viruses in Pineapple in Taiwan. Plant Disease. *93* (*2*) 196. https://doi.org/10.1094/PDIS-93-2-0196C.

Sinha, D.K. and Smith, M. (2014). Selection of Reference Genes for Expression Analysis in *Diuraphis noxia* (Hemiptera: Aphididae) Fed on Resistant and Susceptible Wheat Plants. Nature Reports. 4, 5059 http://doi.org/10.1038/srep05059.

Ventura, J.A, and Zambolim, L. (2002). Controle Das Doenças Do Abacaxizeiro. In Laércio Zambolim; Francisco Xavier Ribeiro do Vale; Ademir José Abranches Monteiro; Hélcio Costa. (Org.). Controle de doenças de plantas frutíferas. *1*, p. 445-509.

Zhou X, Lindsay H, Robinson MD. (2014). Robustly detecting differential expression in RNA sequencing data using observation weights . Nucleic Acids Research. *42* (*11*), e91-10 http://doi.org/10.1093/nar/gku310.

5.2 CAPÍTULO 2

- Capítulo elaborado no formato de artigo para submissão em revista de Qualis A. Os suplementos citados constam como apêndices ao final desta tese.

PMWaV INFECTION PROMOTES SIMULTANEOUS INHIBITION OF UPR PATHWAY AND *RTM2* EXPRESSION IN PINEAPPLE PLANTS

ABSTRACT

Pineapple plants are susceptible to *Pineapple mealybug wilt associated virus* (PMWaV) that causes mealybug wilt pineapple disease. The molecular mechanisms related to this infection have not been reported yet. Thus, in this study, we evaluated the transcriptome of symptomatic and asymptomatic plants to identify biological pathways related to PMWaV susceptibility, and, as a result, its tolerance. Bioinformatics tools were used to analyze the functional classification of differentially expressed genes (DEGs). These analyses indicated the induction of genes associated with floral development and inhibition of genes responsive to abiotic stimuli and biotic stress. In this context, *PRs*, *HSPs*, *WRKYs* and *MYB*s transcription factors were repressed in symptomatic plants, as well as elements regulating unfolded protein response (UPR) and ubiquitin-proteasome system (UPS) pathways. Moreover, there was a significant negative correlation between the levels of *RTM2*, and *HSP20*, and PMWaV-2 transcripts. HSP20 has been reported as a virus movement restriction protein, and it was found accumulated in asymptomatic pineapple plants. The RNA-seq data was confirmed by RT-qPCR for 14 DEGs. This result indicated *RTM2* as a key gene in the regulation of PMWaV-2 infection. In addition, our data report several modulated pathways and point to ER stress as a response to PMWaV infection.

1 INTRODUCTION

Pineapple (*Ananas comosus* var. *comosus*) is typical tropical plant highly appreciated for its flavor, medicinal properties and ornamental potential, being the most cultivated and economically important species of the family *Bromeliaceae* (SOUZA et al., 2012). Moreover, due to its crassulacean acid metabolism (CAM), pineapple has been used as a model for studying the evolution of photosynthesis, showing the interconnection of CAM and the circadian clock (MING et al., 2015). Being a Bromeliaceae and a CAM plant, pineapple is known for its resistance to drought. Nevertheless, a viral disease known as mealybug wilt pineapple (MWP) leads to symptoms correlated to water absorption deficiency and significant losses of production. MWP is characterized by the progression of wilting symptoms that begin with root atrophy, followed by gradual leaf discoloration, reddening, epinasty and aging. Thus, this disease causes changes in the development of pineapple that impair fruit production in quantity and quality, rendering fruits unsuitable for consumption and exportation (MAYO, 2002; VENTURA; ZAMBOLIM, 2002). In addition, controlling the disease in the field by the removal of symptomatic plants is hampered by the presence of asymptomatic infected plants. Although they serve as a source of disease spread through their propagative material, the survival of these plants next to symptomatic plants signals to the development virus tolerance.

MWP is caused by the *Pineapple mealybug wilt associated virus* (PMWaV) complex, comprised by two viruses, PMWaV-1 and PMWaV-2, which belong to the *Ampeloviruses* genus of the *Closteroviridae* family (BORROTO-FERNÁNDEZ; TORRES-ACOSTA; LAIMER, 2007; PERON et al., 2018; SETHER et al., 2005; SHEN et al., 2009). The viruses infect the pineapple phloem cells and can be found in the form of dsRNA in vesicles originating from endoplasmic reticulum or from mitochondria fragmentation inside these cells (AGRANOVSKY, 2016; SETHER; HU, 2002). It has been proposed that proteins encoded by PMWaV viruses can suppress the pineapple RNA silencing defense mechanism (DEY et al., 2015). However, the molecular mechanisms involved in the PMWaV-pineapple interaction were not elucidated yet.

The interaction between plant and viral factors results in the modulation of different defense mechanisms in the plant in order to contain the pathogen at the infection site. In this sense, plants can resist infection by expressing resistance (*R*) genes that trigger a hypersensitive response (HR), or by regulating replication, translation, and cell-to-cell movement of viral particles(MCHALE et al., 2006; PINTO; RIBEIRO; OLIVEIRA, 2011; SHIRASU; SCHULZE-LEFERT, 2000). In this context, reactive oxygen species (ROS) and cross-talk hormonal signal to the expression of pathogenesis-related proteins (PRs), phenolic compounds and phytoalexins, that act on cell wall reinforcement and pathogen containment. Thus, a systemic acquired resistance (SAR) is triggered (BAXTER; MITTLER; SUZUKI, 2014; MCHALE et al., 2006; PINTO; RIBEIRO; OLIVEIRA, 2011; SHIRASU; SCHULZE-LEFERT, 2000; VAN LOON; GERAATS; LINTHORST, 2006). On the other hand, another group of *R* genes, called *RTM* (Restricted Tobacco etch virus Movement) genes, act controlling the dispersion of the *Tobacco etch virus* (TEV) in *Arabidopsis* without activating HR or SAR (CHISHOLM et al., 2001; WHITHAM et al., 2000; YAMAJI et al., 2012). At the level of translational regulation, viruses use cellular machinery and increases the pressure of unfolded proteins on the membrane of the endoplasmic reticulum (ER). To tolerate stress on ER, unfolded protein response (UPR) pathway is activated leading to the expression of chaperones and enzymes involved in ER-associated degradation (ERAD). The prolonged increase in pressure on ER induces changes in UPR that lead to apoptosis (HÜTTNER; STRASSER, 2012; ZHANG; WANG, 2012). The ubiquitination of misfolded proteins and the targeting for degradation by the 26S proteasome in ubiquitin-proteasome system (UPS) requires the participation of chaperones and the recognition of target proteins that can be mediated by auxin and jasmonic acid (JA) (HÜTTNER; STRASSER, 2012; QI et al., 2011; ZHANG; WANG, 2012). In this way, the plant defense mechanisms act in an orchestrated way to determine resistance, tolerance or susceptibility.

The determination of the mechanisms regulated in the PMWaV-pineapple interaction in symptomatic plants may direct plant breeding research with a focus on enhancement of the plant's innate immune response. Thus, our work focused on comparing the transcriptome of the symptomatic and asymptomatic pineapple plants and identifying altered pathways that could confer tolerance or resistance to PMWaV. Recently, we evaluated the transcriptome of PMWaV-2-infected symptomatic

pineapple plants and identified 390 differentially expressed genes (DEGs) (PERON et al., 2018). Here, we evaluated these DEGs through functional enrichment analysis and grouping in categories related to biotic stress. We also validated the expression of 14 genes observed as differentially expressed in RNA-seq data by RT-qPCR. Additionally we investigated the expression of chaperones related to plant-virus interaction in symptomatic plants and sought correlation with PMWaV-2 transcript levels. Moreover, total protein expression of the symptomatic and asymptomatic plant were evaluated by RP-HPLC and the fractions of interest were analyzed by RP-HPLC-MS/MS in order to identify proteins corresponding to candidate genes.

2 METHODS

2.1 PLANT MATERIAL

Pineapple plants (*Ananas comosus* var *comosus*) 'Smooth Cayenne' were cultivated in the experimental field of the Capixaba Institute for Research, Technical Assistance and Rural Extension (INCAPER, Sooretama, ES, Brazil) until the appearance of severe symptoms of MWP (Supplementary Figure SF1). Only plants in the vegetative phase were considered in this study. Based in identification the symptoms in the leaves (wilt, epinasty, and redness), 26 plants were selected and grouped as symptomatic (*n* = 13) and asymptomatic (*n* = 13). In the same proportion, the plants were subdivided in 3 groups for further analysis: (i) RNAseq (*n* = 6), (ii) RNAseq validation (*n* = 10), and (ii) correlation of transcripts levels between PMWaV-2 and chaperones *ERDJ3B*, *RTM2* and *BIP* (*n* = 10) and identification of corresponding proteins. D leaves were collected and frozen in ethanol bath with dry ice. The samples were grounded in liquid nitrogen using a mortar and a pistil until the formation of a thin powder.

2.2 RNA EXTRACTION AND SEQUENCING

RNA extraction was proceeded as described in Peron *et al*, 2018 (PERON et al., 2018). In brief, total RNA was extracted from the basal portion of the leaf (~100 mg) using the mirVana™ miRNA Isolation Kit (Life Technologies, Grand Island, NY, USA). RNA quantity and quality analysis were verified in a Nanodrop 2000 spectrophotometer apparatus (Thermo Fisher Scientific, Waltham, MA, USA). First-strand the cDNA synthesis was obtained using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Grand Island, NY, USA) and PMWaV-1 and PMWaV-2 diagnosis was performed by Peron *et al.*2018. Total RNA (4µg) extracted from biological triplicates of symptomatic and asymptomatic conditions were sent to Macrogen Company (Seoul, South Korea) for Illumina HiSeq 2000.

2.3 BIOINFORMATICS ANALYSIS

In a previous work, we processed pineapple RNA sequencing data obtained by Illumina technology and found 390 DGEs in symptomatic pineapple plants using DESeq2 as described in Peron *et al*., 2018 (PERON et al., 2018). In this work, we evaluated the functional enrichment of these DEGs and grouped them into categories related to biotic stress. Functional annotation of DEGs was obtained using orthologous genes of *Arabidopsis thaliana* TAIR10 recovered using the BioMart tool available in Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html). Functional enrichment analysis was processed with Singular Enrichment Analysis (SEA) using AgriGO tool (http://bioinfo.cau.edu.cn/agriGO/analysis.php). Parameters used in SEA include hypergeometric method adjusted to multiple tests by Yekutieli with a significance level of 0.05 and a minimum of 5 input mappings. Gene ontology (GO) was obtained using the Plant Go slim for Biological Processes, Molecular Function, and Cell Component. In addition, REVIGO tool (http://revigo.irb.hr/) was used to summarize lists of GO terms. Pathway analysis was performed using MapMan v.3.6.0 0 tool (http://mapman.gabipd.org) to identify over-represented cellular functions in response to biotic stress. Protein interaction network was predicted using the STRING database V 10.5 (https://string-db.org) to visualize the interaction of the gene products of the DEGs in the enriched biological processes. DEGs were selected for validation with RT-qPCR based on the functional classification and enriched biological processes and the involvement with the plant-virus interaction.

2.4 VALIDATION DEGs BY RT-qPCR

The relative expression of the 14 selected DEGs was estimated with specific primers to the genic loci of *A. comosus* v3. Design and viability of the primers were evaluated using the PrimerQuest and OligoAnalyzer tools (https://www.idtdna.com). The melting curve was obtained for all primers with only a single peak confirming the synthesis of a single product. A total of 10 µl of a reaction solution containing 10 ng of cDNA, 5 µl SYBER Green® PCR Master Mix (Applied Biosystems, Carlsbad, USA) and gene primers at the concentration of 10 µM each primer were used (Supplementary Table ST3). Reactions were performed on a 7500 Fast Real-time

PCR system version 2.0.1 (Applied Biosystems, Carlsbad, USA). qPCR conditions were 20 s at 95°C, followed by 40 cycles of 3 s at 95 °C, and 30 s at 60°C, followed by incubation at 95°C for 15 s, 60°C for 60 s and 95°C for 15 s for melting curve detection. Relative expression was estimated by $2^{-\Delta\Delta Ct}$ method, using the gene coding for actin as housekeeping to normalize the target genes expression levels (LIVAK; SCHMITTGEN, 2001). Samples were amplified in technical duplicate and the average d*C*t values were used for the calculation of the relative expression each target gene. The statistical significance of the expression values presented as $\log_2$ fold changes was evaluated by t-test with standard error calculations between the groups of pineapples plants. Statistical analyzes were carried out using GraphPad Prism statistical software (La Jolla, California, USA).

2.5 TOTAL PROTEIN EXTRACTION

A volume of 2mL of 0.1mol.L-1 potassium phosphate buffer pH 6.8 was added to 300 mg of the powder and the mix was centrifuged at 10,000 g for 30 min at 4 ° C (BETTINI et al., 2014). The supernatant of crude extract was collected and protein quantification was estimated according to the method described by Bradford (BRADFORD, 1976), using bovine serum albumin as standard. To each condition, a pool containing 300 µg of crude extract proteins was prepared by mixing 60 µg of protein from each biological replicate. The protein extracts pools were lyophilized and stored at -20º C for further analysis.

2.6 DETERMINATION OF PROTEIN PROFILE AND THE RELATIVE MOLECULAR MASS OF THE NATIVE PROTEIN BY SIZE EXCLUSION CHROMATOGRAPHY

The protein profile determination of plant extract was obtained by size exclusion chromatography using an Ultra Hydrogel TM 250 Waters column (7.8×300 mm) coupled to a HPLC System (Prominence of Shimadzu). For protein profile and relative molecular mass determination, samples solution of 10 µL (1.7 mg mL^{-1}) were loaded on to column. The mobile phase was tris-HCl 100 $mmol.L^{-1}$ at pH 7.5 at flow rate of 0.8 mL min^{-1} and the eluate was monitored by ultraviolet absorption at 280

nm. The analysis was performed thrice. The molecular mass standards used for column calibration were β-amylase (205 kDa); alcohol dehydrogenase (150 kDa); bovine serum albumin (66 kDa); ovalbumin (46 kDa); trypsinogen (25 kDa) and cytochrome *C* (12.5 kDa). The retention volume of standards were used for calculation of K_{av} and this values ploted as a function of the logarithm of molecular mass, and relative molecular masses were calculated by equation derived from previous plot.

2.7 PROTEIN IDENTIFICATION

Protein identification was performed using lyophilized protein crude extract of symptomatic and asymptomatic plant. Samples were suspended in 4 mL of water and centrifuged at 12,000 rpm for 5 minutes. Separation was performed by RP-HPLC using the Teknokroma SEA 18 column (250 x4 mm 5 μm of particle size) loaded with 500 μL of each plant condition. The elution was performed a flow rate of 500 μL.min^{-1} using a linear gradient of acetonitrile (5-90% in 70 minutes), containing 0.1% v/v trifluoroacetic acid and monitored at 214 nm. Fractions were collected, vacuum dried, and stored at −20 °C until use. The samples were suspended in amonium bicarbonate 25mM and tryptic protein digestion were conducted in accord of Ferreira et al., 2016 (FERREIRA et al., 2016). Electrospray tandem mass spectra were recorded using a Q-Tof quadrupole/orthogonal acceleration time-of-flight spectrometer (Waters, Milford, MA) interfaced to the Nano acquity system capillary chromatograph in positive íon mode, and the peptide spectra were compared with sequences in the *Ananas comosus* Uniprot database (release april_2018) via MASCOT software searches (version 2.2.04). Peptides matching contaminants (keratin and trypsin) were excluded.

3 RESULTS

3.1 TRANSCRIPTOMIC ANALYSIS

In a previous study, the transcriptome analysis of *A. comosus* revealed a list of 16,097 expressed genes, where 390 are differentially expressed in the symptomatic condition of MWP with regard to the asymptomatic plants (PERON et al., 2018). In this work, we further evaluated these DEGs (122 up-regulated genes and 268 down-regulated; Supplementary Table ST1) through functional analysis. Cell functions of gene products were determined mapping DEGs in Mapman functional categories (Figure 1, and Supplementary Table ST2).

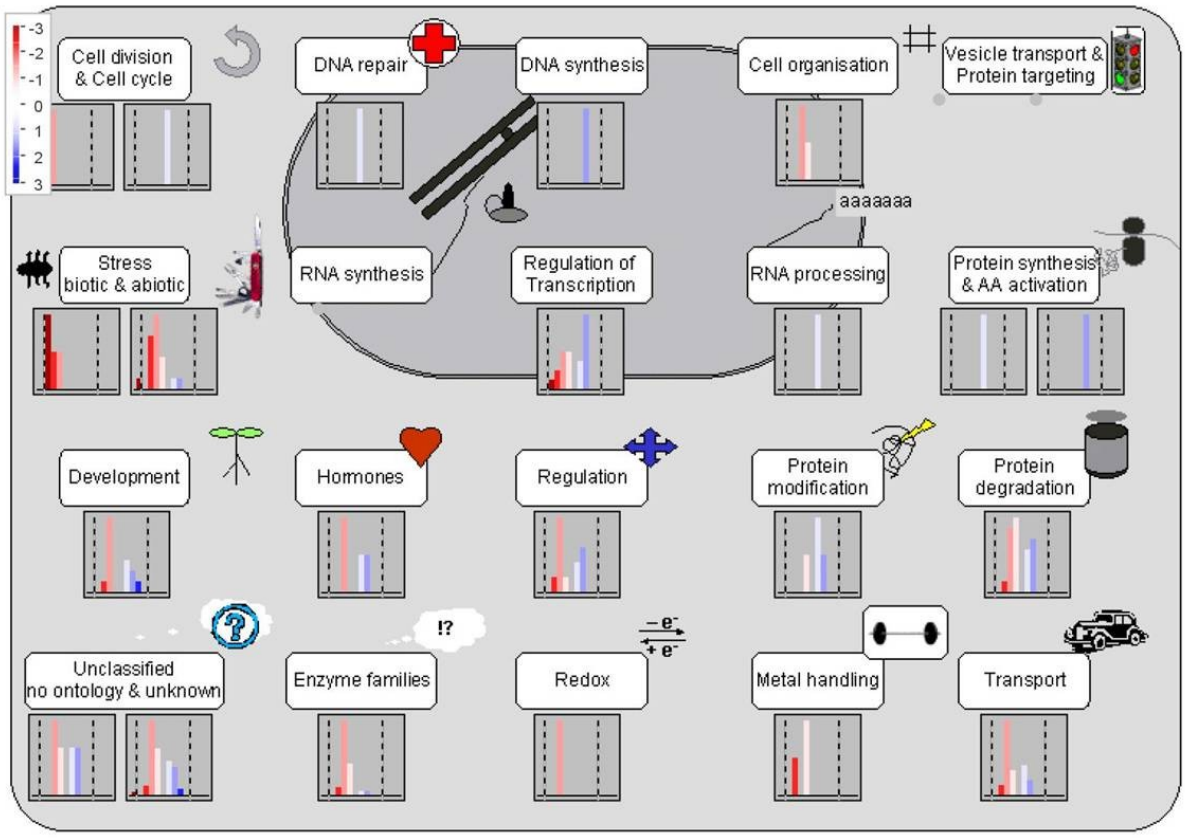


Figure 1 Mapping of DEGs in cell function overview using Mapman. Heat map represents the fold change revealed by the analysis with DESeq2. The red color indicates the down-regulated genes and blue indicates up-regulated.

Enzyme families, *transport*, *protein degradation*, *abiotic and biotic stress* and *regulation of transcription* were the functions most represented. These functions

group repressed genes encoding heat shock proteins (HSPs) (BIP, DNAJ and HSP21), the family of the lipase GDSL enzymes (GDSL-motif lipase/hydrolase family protein), glutathione S transferases (GSTFs), peroxidases, and transporters (water channel, sugars, amino acids, metabolites and ion transporters). Among the functions most represented by induced genes are regulation of the response to auxin by Auxin Response Factor (ARF) (ARF2, ARF4, and ARF11) and protein degradation via SCF with F-box protein (ZTL, etc.). A considerable portion of the DEGs mapped on *biotic stress* were attributed to *proteolysis* (27%), to *abiotic stress* (17%), to *signaling* (11%), and to the *cell wall* (10%) (Supplementary Table ST2). In this context we observed negative regulation of PRs (PR3, PR4, and PR5); TF type WRKY (WRKY40, WRKY51, and WRKY33), and MYB (MYB93, and MYB86). Inhibition of the secondary metabolites (LAC4, LAC11, LAC12, etc.), ubiquitin-like protein (SUM2), and zinc finger (C3HC4-type RING finger) family proteins, and hormonal regulation (jasmonate metabolism: LOX5 and AOS; auxin metabolism: SAUR_B and ILL6) were also observed. On the other hand, genes encoding ubiquitin-containing protein (UBC1 and UBC19) were induced.

To improve the analysis of the biological pathways most affected by MWP disease, we used AgriGO singular enrichment analysis (SEA) and mapped DEGs in the categories biological processes, molecular function, and cellular component. GO enrichment analysis showed biological processes related to plant defense repressed while the development and reproductive processes were induced (Figure 2A-B). Among the down-regulated genes, the most represented biological processes were *response to stress* (GO: 0006950) and *response to abiotic stimulus* (GO: 0009628); and the most represented cell components were *extracellular region* (GO: 0005576), *vacuole* (GO: 0005773), and *membrane* (GO: 0016020) (Figure 2C). Among the up-regulated genes, the most represented biological processes were *flower development* (GO: 0009908), *post-embryonic development* (GO: 0009791) and *reproduction* (GO: 0000003); and the most represented molecular functions were *signal transducer activity* (GO: 0004871), *molecular transducer activity* (GO: 0060089), and *protein binding* (GO: 0005515) (Figure 2B-D).

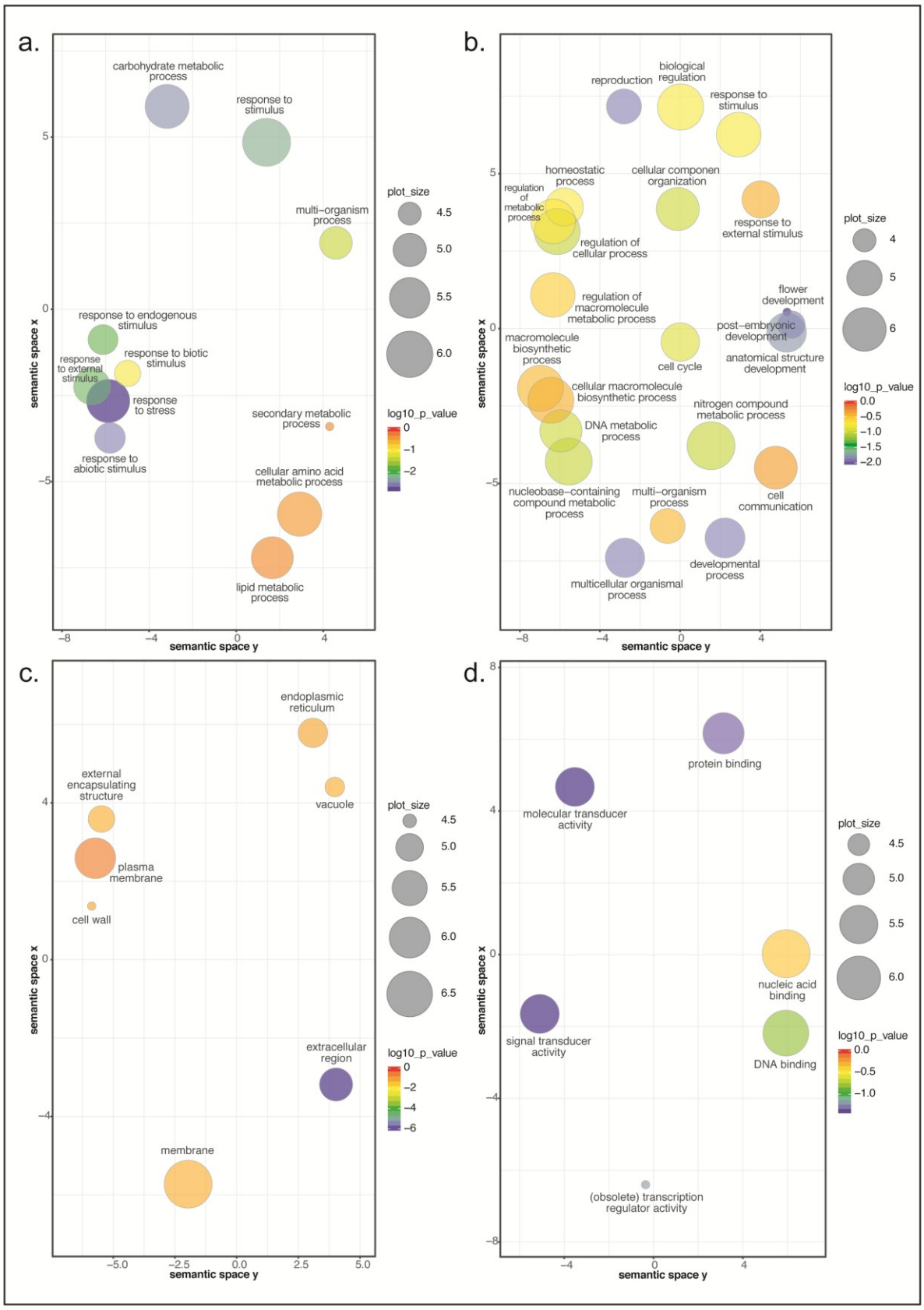


Figure 2 Visualization with Revigo. GO terms (Plant Go Slim) significantly enriched for biological processes in *Ananas comosus* cv Smooth Cayenne according to down-regulated (A) and up-

regulated (B) genes. In (C), Go enriched terms for Cell Component according to down-regulated genes. In (D), Go terms enriched for Molecular Function according to the up-regulated genes. Terms were grouped by semantic similarity and are represented by adjacent circles being strictly related as close they are. Size of circle indicates the frequency of GO term and color refers to a log10P value resulting from the enrichment, with the gradient from blue to red proportional to the values in ascending order.

STRING was used to visualize the interaction of gene products of the 339 DEGs that presented protein homology to *Arabidopsis* proteins in the STRING database. A protein interaction network of 339 nodes with 415 edges was obtained (Figure 3). We identified clusters for TFs associated to hormonal regulation by auxin (ARFs, as ARF4, and AUX/IAA, as SHY2) and JA (TIFY10b), besides WRKYs (WRKY39, WRKY33, WRKY40, and WRKY51) and MYBs (MYB93 and MYB86). In addition, clusters related to jasmonate synthesis (AOS and LOX); regulation of homeostasis by aquaporins (PIPs, DELTA-TIP, GAMMA-TIP and NIP5); PRs associated with the SAR pathway (OSM34, PR4 and CHIA); pectinase, laccases, peroxidases and galactosidases involved in cell wall maintenance (AGALs, QRTs, HCHIB, ESK1 among others); elements of the ubiquitin proteasome pathway (UBC1, UBC19, SUMO2, RHF2A, ZTL among others); and stress responsive proteins such as calmodulin (CML42 and CAM5), calreticulin (CRT1b), heat shock protein (HSPs) (RTM2 and ERdj3B). In addition, BiP and IRE1 key elements in the unfolded protein response (UPR) pathway to stress response were observed (ZHANG; WANG, 2012).

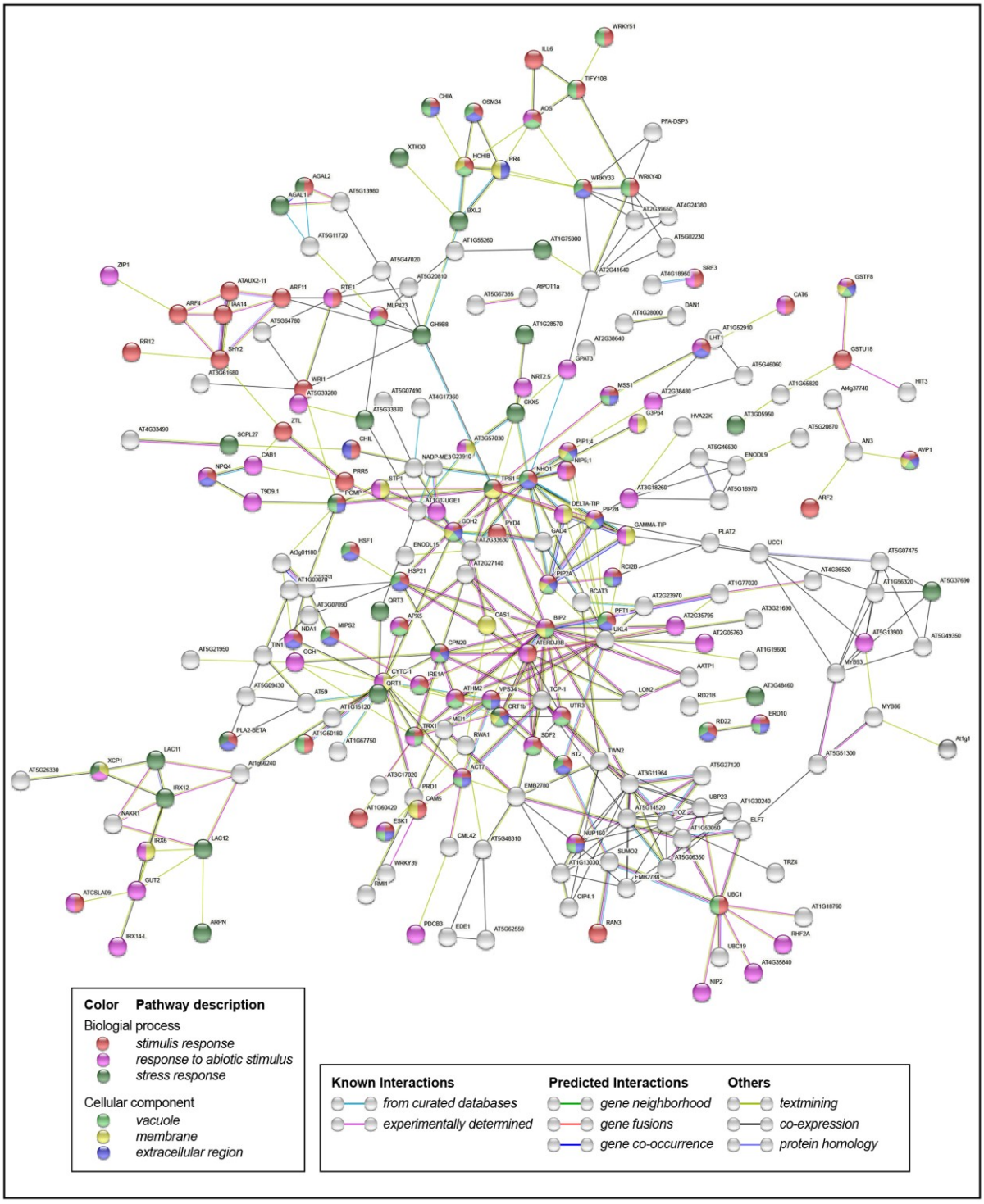


Figure 3 Interaction network built on STRING for DEGs using evidence of interaction and medium confidence as parameters. The gene clustering under GO terms was color-labeled.

3.2 VALIDATION OF CANDIDATION GENES BY RT-qPCR

RT-qPCR validation was performed for 14 DEGs (Table 1) in asymptomatic and symptomatic infected plants (Figure 4A-B).

Table 1 - DEGs detected through RNA-seq analysis and selected for validation (PERON et al., 2018) by RT-qPCR.

ID *A.comosus*	Log2 FC	ID TAIR10	Gene	Description (Phytozome, TAIR10 or NCBI database)
Aco008670	-4,12	AT4G11650	OSM34	Osmotin 34 / thaumatin-like protein
Aco023684	-3,16	AT1G74950	JAZ2	TIFY domain/jasmonate-zim-domain protein 1
Aco004215	-2,97	AT3G04720	PR4	Pathogenesis-related 4
Aco016970	-2,27	AT3G07600	Aco016970	NBS-LRR class disease resistance protein
Aco005912	-2,1	AT2G27140	RTM2	HSP20-like chaperones superfamily protein
Aco007632	-2,01	AT5G42020	BIP	Heat shock protein 70 (Hsp 70) family protein
Aco011802	-1,45	AT2G47730	GSTF8	Glutathione S-transferase phi 8
Aco008411	-1,11	AT5G11720	SHY2	AUX/IAA transcriptional regulator family protein
Aco007383	-0,99	AT3G62600	ERDJ3B	DNAJ heat shock family protein
Aco012406	-0,93	AT1G18650	PDCB3	Plasmodesmata callose-binding protein 3
Aco018061	1,23	AT5G60450	ARF4	Auxin response factor 4
Aco016766	1,29	AT5G24470	PRR5	Pseudo-response regulator 5
Aco001358	1,36	AT1G05010	ACO4	1-aminocyclopropane-1-carboxylate oxidase
Aco013232	1,37	AT1G79730	ELF7	Hydroxyproline-rich glycoprotein family protein

BIP, ERdj3B and RTM2 were chosen because they are expressed in response to different stresses and are related to the regulation of viral infection in plants (HÜTTNER; STRASSER, 2012; WHITHAM et al., 2000; ZHANG; WANG, 2012). Likewise, Aco016970, that codes for a protein with the NBS-LRR domain (ATG07600). NBS-LRR comprises the major class of *R* genes and encoding nucleotide-binding leucine-rich proteins in response to avirulence (*Avr*) gene expression of the pathogen trigger HR with oxidative stress (MCHALE et al., 2006). *PR4, PDCB3*, and *OSM34* respond to biotic stress via the SAR pathway. *ARF4, SHY2, TIFY10B, ACO4*, and *ELF7* are involved in the possible auxin/JA/ET cross-talk. In addition, *PRR5* was evaluated because of its importance in the regulation of the pineapple's cell cycle (ZHANG; LIU; MING, 2014b) and *GSTF8* due to its

association with the redness symptom in GLRaV-3-infected vines (ESPINOZA et al., 2007). All the genes analysed by qRT-PCR presented the same expression profile as observed in RNA-seq (Pearson correlation coeficient = 0,788) (Figure 4A, and 4C).

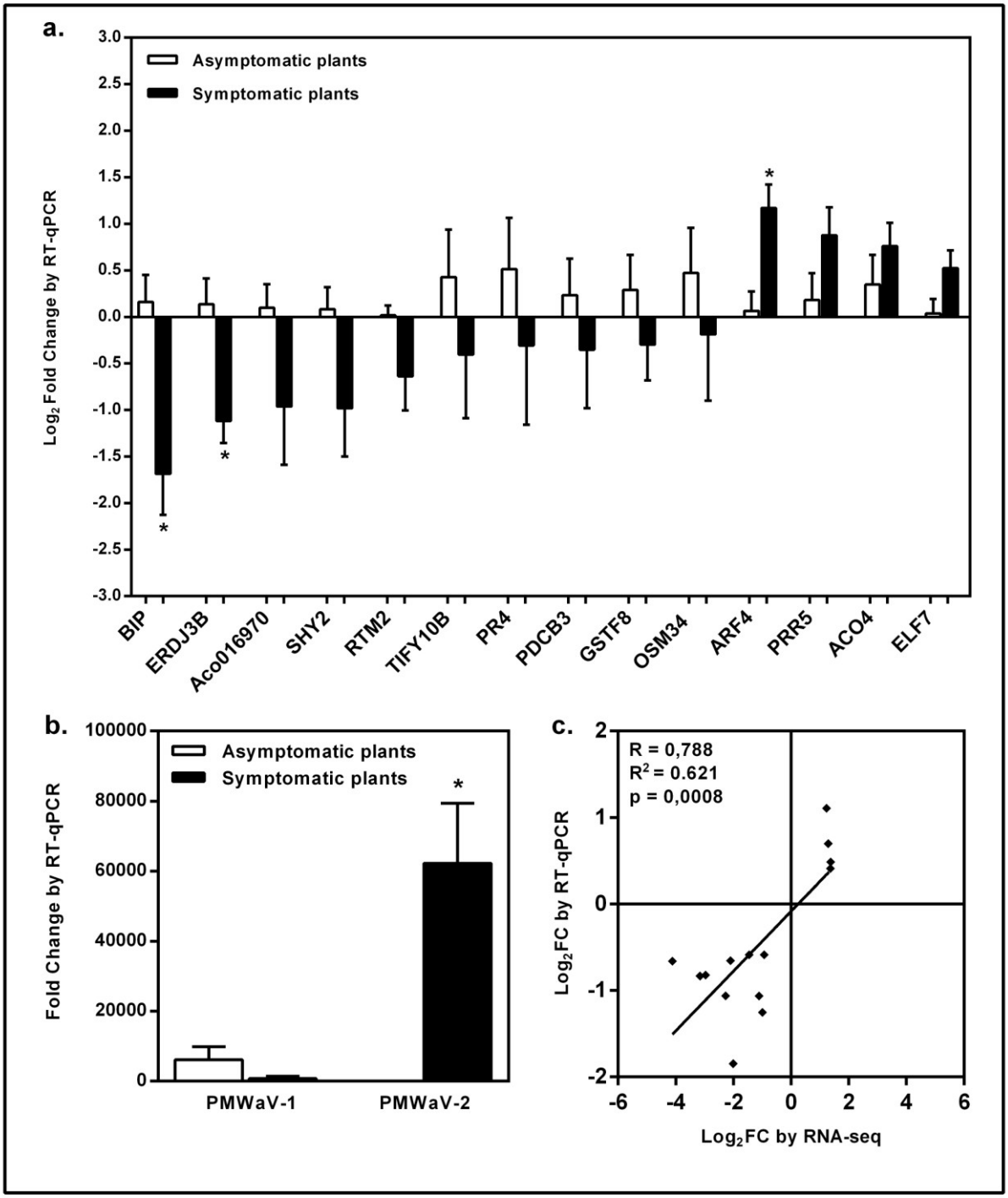


Figure 4 RT-qPCR validation of 14 DEGs (A) and determination of transcript levels of PMWaV-1 and PMWaV-2 (B) in symptomatic (*n* = 5) vs. asymptomatic (*n* = 5) pineapple plant groups. (C) Pearson correlation between the expression values detected by RNA-seq and qRT-PCR for the genes tested. Bar represent mean +/- standard error. Asterisks indicate significant differences evaluated by Tukey test ($p < 0.05$).

3.3 INVESTIGATION OF THE RELATIONSHIP BETWEEN CHAPERONES AND PMWaV

The gene expression of some important chaperones was repressed in response to PMWaV infection in symptomatic pineapple plants. Among them, BiP (HSP70) plays a key role in the control of ER quality and is helped by ERdj3B (HSP40) that is also repressed. Changes in the expression of these chaperones are related to UPR pathway and responses to viral infections at the local level. In addition to these HSPs, *RTM2,* that encodes an HSP20, has also been repressed. This gene is associated with the control of long-distance mobility of the TEV virus in *Arabidopsis* phloem cells (WHITHAM et al., 2000). Given the same nature of PMWaV virus dispersion, we investigated the expression of *RTM2,* in addition to *BiP* and *ERdj3B,* in symptomatic pineapple plants and correlated with the transcript levels of PMWaV-2. The result confirmed the inhibition of the expression of these chaperones although only *RTM2* was significantly inhibited ($p < 0.05$) (Figure 5A).

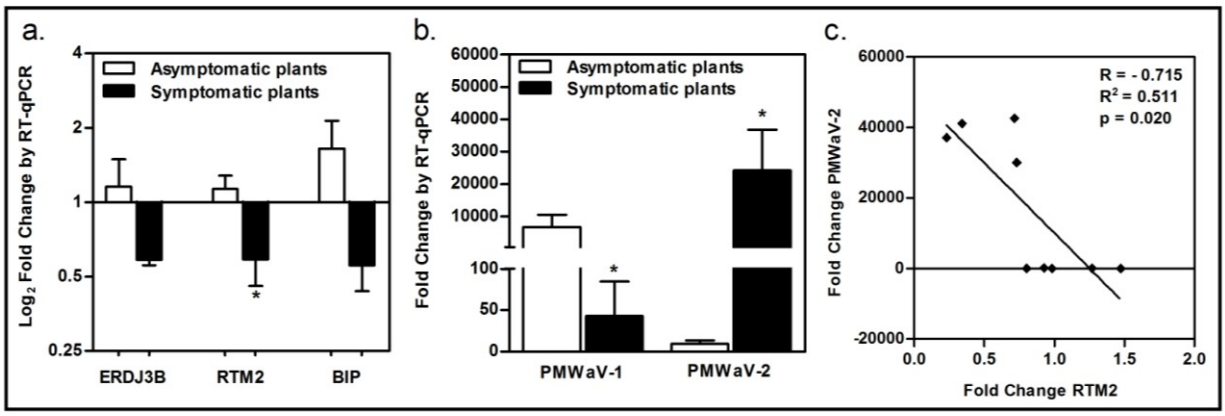


Figure 5 Relative expression of chaperones (A) and of PMWaV-1 and PMWaV-2 (B) by RT-qPCR in symptomatic (n = 5) and asymptomatic (n = 5) pineapple plant group. (C) Pearson correlation between the expression values of the PMWaV-2 and *RTM2*. Bar represents mean +/- standard error. Asterisks indicate significant differences evaluated by Tukey test ($p < 0.05$).

Plants with symptoms showed levels of PMWaV-2 transcripts higher than those found in asymptomatic plants, although both were infected (Figure 5B). Thus, when correlating the levels of *RTM2* transcripts with the levels of PMWaV-2 transcripts, we obtained a considerable negative correlation (R = -0.715) (Figure 5C). To *ERdj3B*

and *BiP*, the Pearson correlations were -0.45 and -0.44, respectively. Therefore, in plants infected by PMWaV-2, inhibition of *RTM2* occurs simultaneously with an increase in mRNA levels of the PMWaV-2.

3.3 IDENTIFICATION OF PROTEINS IN UPR PATHWAY AND HSP20 (PUTATIVE RTM2)

A similar protein profile separation was obtained by size-exclusion chromatographic of the symptomatic and asymptomatic conditions of MWP (Figure 6).

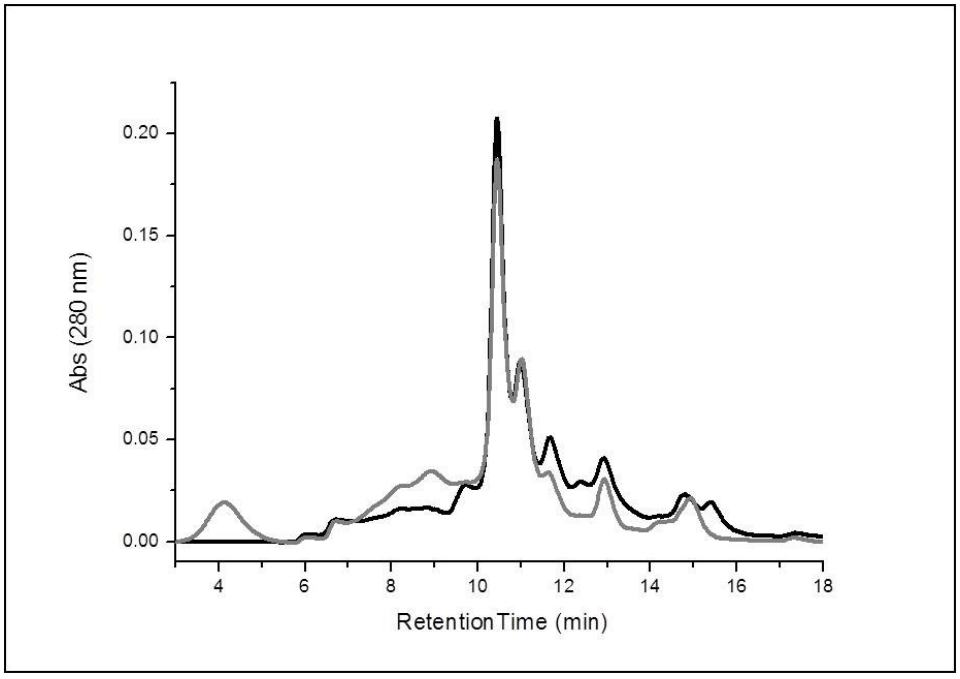


Figure 6 Protein profile by size exclusion chromatography of symptomatic (black line) and asymptomatic (gray line) pineapple plant.

The chromatographic process was able to separate molecules into three bands as function of retention time (RT), large (6.5- 10.2 min; 8.4-251 kDa), (10.2 - 12.5 min; 0.9-8.4 kDa), and small (12.5 - 13.7 min; 0.3-0.9 kDa) and very large (RT < 5 min; > 1.10^3 kDa) molecular weight. Although of similar profiles, protein concentration in the range of 6 to 9.5 min was significantly higher for the asymptomatic plant. The identification of proteins present in this fraction was conducted by mass

spectrometry, only identifications with ion score sufficient to indicate the identity (with significance of p <0.05 according to the software algorithm) were considered, and only proteins identified with at least one exclusive peptide were considered in the analysis. Asymptomatic plants displayed accumulation of proteins associated with cell wall reinforcement, such as Fasciclin-like arabinogalactan protein 2 (*m* = 27.015 Da), Fasciclin-like arabinogalactan protein 2 (*m* = 36.623 Da), Fasciclin-like arabinogalactan protein 9 (*m* = 43.614 Da), and Plasmodesmata callose-binding protein 3 (*m* = 20,808 Da). In addition, asymptomatic but not symptomatic plants accumulated chaperones (17.9 kDa class II heat shock protein, *m* = 18.029 Da; Calreticulin, *m* = 46.561 Da; and Heat shock 70 kDa protein 14, *m* = 94.682 Da); of Calmodulin, *m* = 16.894 Da, Small ubiquitin-related modifier (*m* = 11.087 Da), and Tetrameric ubiquitin (*m* = 34.152 Da).

4 DISCUSSION

The severity of symptoms of MWP disease results from root death and leaf wilting, which lead to the fruiting difficulty for the plant. In this context, the analysis of DEGs revealed inhibition of genes responsive to biotic and abiotic stresses while induced genes were associated with alteration of the developmental phase. Although asymptomatic and symptomatic infected plants are clones at the same developmental phase and have received the same care, many genes encoding transmembrane transporters have been repressed in the diseased plants, including those coding for aquaporins (AQPs) (*DELTA-TIP*, *GAMMA-TIP*, *PIP1;4*, and *PIP2;A*), copper (AT3G07600) and zinc (*ZIP1*) transporters. The participation of AQPs in the influx of water from growing tissues under irrigation conditions was reported in *Setaria viridis* and *Solanum lycopersicum* (MCGAUGHEY et al., 2016). In addition, transported minerals, copper and zinc, participate in detoxification of superoxide radicals by the enzymes Cu-and Zn-superoxide dismutase, which protect cells from oxidative damage. The reduction in the transport of these minerals interferes in the fixation of CO_2 and its derivatives, altering the membrane composition (CAKMAK, 2000). Copper shortage also impairs cell wall lignification by loss of activity in reducing phenol transformation. In addition, the expression of *FLA13* that encoding

fasciclin-like arabinogalactan protein 13 was suppressed, which in *Arabidopsis* acts positively on cell wall biosynthesis (SEIFERT; XUE; ACET, 2014). Also, FLA2, FLA9 and FLA11 proteins were identified only in asymptomatic pineapple plants. Reduced expression of transmembrane transporters may be associated with wilt symptoms such as loss of turgidity.

In response to oxidative stress caused by viral infection, the plants activate the SAR pathway expressing pathogenesis-related proteins (PRs) with chitinase-like properties, including *PR3*, *PR4*, and *CHIA*, in addition to the *PDCB3* glucanase. However, the expression of these enzymes was down-regulated in pineapple with MWP symptoms, which makes it difficult to contain the virus in infection site. Rice plants genetically modified to overexpress *PR4* showed tolerance to drought, pathogens, salt stress, cold, injury, thermal shock and UV radiation (WANG; XIAO; XIONG, 2011). In addition, *osmotin34* (*PR5*), a gene associated with water deficit in plants (SINGH; MAKKAR; NEGI, 1989), was also down-regulated. Thus, PMWaV infection interfered with the plant's ability to maintain water and osmotic balance leading to the wilt symptom both by altered aquaporin expression and by the difficulty in maintaining cell wall integrity. This is also because ROS-removing enzymes and cell wall protectors, such as peroxidases, laccases, thioredoxins, and GSTF, were also down-regulated in plants with symptoms of MWP. Thus, the defense mechanisms associated with SAR was repressed.

The regulation of stress responses and activation of the SAR pathway is modulated mainly by ROS and hormonal cross-talk. MWP disease reduced AJ mediated responses both by repression of AJ biosynthesis genes (*AOS* and *LOX*) and by repression of responsive TFs (*WRKY51*, *WRKY33*, *WRKY40,* and *TIFY10B*). The expression of WRKY and MYB TFs is required for virus resistance, as observed in the interactions between *Barley yellow mosaic virus* and *Brachypodium distachyon* (WANG et al., 2017) and in the Citrus-*Citrus tristeza virus* interaction (CTV) (FU et al., 2016). On the other hand, we observed the positive regulation of auxin responses with the induction of ARFs and inhibition of AUX/IAA repressors TFs. ARF4, for example, is an auxin-induced TF that regulates the stomatal movement in CAM (ABRAHAM et al., 2016) and has been associated with the development of carpels during the development of floral organs (FINET et al., 2010). Auxin homeostasis also

is proposed as a point of defense regulation of plants against the virus (MAYDA et al., 2000). The imbalance may induce morphological changes such as foliar distortion (PENNAZIO; ROGGERO, 1996; PÉRET et al., 2012) and may help to explain the occurrence of symptoms in plants with MWP. Positive regulation of auxin efflux was observed in vines in response to GLRaV-3 (ESPINOZA et al., 2007), a representative virus of the same genus as PMWaV. In *Arabidopsis*, it was observed that *Tobacco mosaic virus* (TMV) interacts with the AUX/IAA PAP1 protein, a regulator of auxin responsive gene expression, with consequent loss of apical dominance as a symptom of the disease (PADMANABHAN et al., 2005). Thus, an antagonistic cross-talk between AJ and auxin may be associated with increased tolerance to PMWaV, development MWP symptoms and reproductive process.

Modulation of the transition from the vegetative to the reproductive development phase in pineapple is definitive and regulated by ethylene. The key enzyme for the production of this hormone, 1-aminocyclopropane-1-carboxylate oxidase, had the gene expression induced in the symptomatic plants, revealing the regulation of flowering (NEKRASOV et al., 2009; REDWAN; SAIDIN; KUMAR, 2016; SCHOTT et al., 2010; TRUSOV; BOTELLA, 2006). Other genes encoding flowering regulators in pineapple were also induced: *PRR5*, essential for circadian rhythm oscillation (ZHANG; LIU; MING, 2014b), and *ELF7,* which acts on the epigenetic control of flowering in the absence of vernalization (HE; DOYLE; AMASINO, 2004; LIU; FAN, 2016). In *Arabidopsis,* transgenic plants that overexpress *PRR5* presented an altered circadian rhythm, with consequent early flowering and red light sensitivity (NAKAMICHI et al., 2012). In addition, in CTV-infected Citrus was observed the up-regulation of the *PRR5* (FU et al., 2016). Therefore, PMWaV infection induced the expression of flowering regulatory elements of in pineapple.

PMWaVs are detected in phloem cells, where they move for long distances. To date, there are no known mechanisms in pineapple that could limit this displacement. However, in *Arabidopsis*, the expression of chaperone RTM2 (HSP20) interferes in the dispersion of *Tobacco etch potyvirus* (TEV) (WHITHAM et al., 2000). Like TEV, PMWaV-2 is a positive RNA virus that moves systemically through the phloem. In plants with symptoms of MWP, we observed a significant negative correlation between levels of *RTM2* and PMWaV-2 transcripts, the main virus associated with

symptoms. In addition, we report the presence of HSP20 protein in asymptomatic plants that were not identified in the symptomatic samples. Thus, we believe that the modulation of *RTM2* expression may regulate the dispersion of PMWaV-2 and, consequently, delay or prevent systemic infection.

In addition to *RTM2*, other genes that encoding chaperones involved in ER quality control were repressed, although plant viruses depend on plant chaperones for the conformation of their proteins. In response to GLRaV-3 infection, vine represses the expression of HSP70 (ESPINOZA et al., 2007). In pineapple plants with MWP symptoms, the same was observed for *BiP,* an *HSP70* with a central function in the UPR. In the initial conditions of stress, the BiP is hijacked for modulation of unfolded proteins, dissociating itself from protein signals. When the dissociated protein is IRE1, a signaling cascade occurs and results in the expression of more chaperones. In this way, the UPR route attempts to meet the need for more chaperones to maintain the translation machinery or redirects the cell to apoptosis when misfolded protein accumulation occurs (HÜTTNER; STRASSER, 2012; MAULE; LEH; LEDERER, 2002; PARK; SEO, 2015; ZHANG; WANG, 2012).

The function of BiP is regulated by association with proteins of the HSP40/DnaJ family, including ERdj3B. Luminal proteins, such as SDF2, form a stable complex with ERdj3 that mediates the interaction of BiP with poorly folded proteins to prevent aggregation and degradation. In *Arabidopsis*, the *SDF2* expression is induced in response to stress. The loss of SDF2 function has been shown to lead to the degradation of leucine-rich transmembrane receptor kinases (LRR-RKs) that recognize pathogen (NEKRASOV et al., 2009). Furthermore, it is believed that DNAJ chaperone participates in the synthesis of negative-strand RNA to the BMV virus (TOMITA et al., 2003). In GLRaV-3-infected vines, it is proposed that plants have repressed DNAJ as a strategy to contain viral replication (ESPINOZA et al., 2007). Thus, negative regulation of the gene expression of the *SDF2-BiP-ERdj3B* complex and positive regulation of the *IRE1-2* that were observed in symptomatic pineapple plants with MWP may be related to PMWaV susceptibility (Supplementary Table ST1). In addition to the HSPs, ER-resident chaperones encoded by *CRT2* (calreticulin) and associated protein encoded by *CAM5* (calmodulin) were also repressed (Supplementary Table ST1). The calreticulin, calmodulin, and heat shock

protein 70 proteins were detected only in asymptomatic plants, confirming the regulation of the UPR pathway in plants with MWP. Thus, misfolded proteins that would normally be directed to ubiquitination and subsequent degradation by the proteasome in the ERAD pathway, increase the pressure on the ER causing stress (HÜTTNER; STRASSER, 2012; ZHANG; WANG, 2012). In symptomatic pineapple plants, this pathway was also negatively modulated by the repression of genes encoding E3 ubiquitin-protein ligase and ubiquitin-like superfamily protein, involved in UPS (Supplementary Table ST2). Thus, our data point to changes in the processes of maintenance of cellular homeostasis mediated by the UPR and ERAD pathways with possible targeting for apoptosis and alteration of pineapple development phase.

5 CONCLUSION

MWP disease interferes with plant immunity, repressing the SAR, UPR and ERAD pathways. Differently from what occurs in infections with activation of *RTM* genes, in MWP, modulation of both HR and SAR occurs as well as the expression of *R* genes. In this sense, we report the relationship between the transcripts levels of *RTM2* and PMWaV-2 as a source of study for the control of this infection. In addition, it is possible that the expression of *HSP40 (ERDJ3B)* and *HSP70 (BIP)* is repressed by the plant as a strategy of tolerance to PMWaV-2, as reported for vines infected by GLRV-3 (ESPINOZA et al., 2007). With prolonged exposure to these events, an apoptosis response can be triggered by IRE1. Concomitantly, hormonal regulation was observed in response to auxini and AJ associated with the development of symptoms. Thus, our work contributes to the study of Mealybug wilt of pineapple and indicates pathways to improve the plant's defenses.

7 REFERENCES

1 ABRAHAM, P. E. et al. Transcript, protein and metabolite temporal dynamics in the CAM plant Agave. **Nature Plants**, v. 2, n. 12, p. 16178, 2016.

2 AGRANOVSKY, A. A. Closteroviruses: Molecular Biology, Evolution and Interactions with Cells. In: **Plant Viruses: Evolution and Management.** Singapore: Springer Singapore, p. 231–252, 2016.

3 BAXTER, A.; MITTLER, R.; SUZUKI, N. ROS as key players in plant stress signalling. **Journal of Experimental Botany**, v. 65, n. 5, p. 1229–1240, 2014.

4 BETTINI, M. O. et al. Enzimas antioxidativas em tecidos vegetais. In: BROETTO, F. (Ed.). **Métodos de trabalho em bioquímica vegetal e tecnologia de enzimas**. Botucatu. p. 23–28, 2014.

5 BORROTO-FERNÁNDEZ, E. G.; TORRES-ACOSTA, J. A.; LAIMER, M. RT-PCR detection and protein-protein interaction of viral components of pineapple mealybug wilt-associated virus 2 in Cuba. **Journal of Plant Pathology**, v. 89, p. 435–439, 2007.

6 BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, n. 1–2, p. 248–254, 976.

7 CAKMAK, I. Role of Zinc in Protecting Plant Cells from Reactive Oxygen Species. **New Phytologist**, v. 146, n. 2, p. 185–205, 2000.

8 CHISHOLM, S. T. et al. Arabidopsis RTM1 and RTM2 Genes Function in Phloem to Restrict Long-Distance Movement of Tobacco Etch Virus. **PLANT PHYSIOLOGY**, v. 127, n. 4, p. 1667–1675, 2001.

9 DEY, K. K. et al. Analysis of pineapple mealybug wilt associated virus -1 and -2 for potential RNA silencing suppressors and pathogenicity factors. **Viruses**, v. 7, n. 3, p. 969–995, 2015.

10 ESPINOZA, C. et al. Gene expression associated with compatible viral diseases in grapevine cultivars. **Functional & Integrative Genomics**, v. 7, n. 2, p. 95–110, 2007.

11 FERREIRA, F. R. B. et al. Evaluation of antimicrobial, cytotoxic, and hemolytic activities from venom of the spider Lasiodora sp. **Toxicon**, v. 122, p. 119–126, 2016.

12 FINET, C. et al. Parallel structural evolution of auxin response factors in the angiosperms. **The Plant Journal**, v. 63, n. 6, p. 952–959, 2010.

13 FU, S. et al. Transcriptome analysis of sweet orange trees infected with 'Candidatus Liberibacter asiaticus' and two strains of Citrus Tristeza Virus. **BMC Genomics**, v. 17, n. 1, p. 349, 2016.

14 HE, Y.; DOYLE, M. R.; AMASINO, R. M. PAF1-complex-mediated histone methylation of FLOWERING LOCUS C chromatin is required for the vernalization-responsive, winter-annual habit in Arabidopsis. **Genes & Development**, v. 18, n. 22, p. 2774–2784, 2004.

15 HÜTTNER, S.; STRASSER, R. Endoplasmic Reticulum-Associated Degradation of Glycoproteins in Plants. **Frontiers in Plant Science**, v. 3, 2012.

16 LIU, C.; FAN, C. De novo Transcriptome Assembly of Floral Buds of Pineapple and Identification of Differentially Expressed Genes in Response to Ethephon Induction. **Frontiers in Plant Science**, v. 7, p. 203, 2016.

17 LIVAK, K. J.; SCHMITTGEN, T. D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. **Methods**, v. 25, n. 4, p. 402–408, 2001.

18 MAULE, A.; LEH, V.; LEDERER, C. The dialogue between viruses and hosts in compatible interactions. **Current Opinion in Plant Biology**, v. 5, n. 4, p. 279–284, 2002.

19 MAYDA, E. et al. Expression of a Pathogen-Induced Gene Can Be Mimicked by Auxin Insensitivity. **Molecular Plant-Microbe Interactions**, v. 13, n. 1, p. 23–31, 2000.

20 MAYO, M. A. Virus taxonomy. **Archives of virology**, v. 147, n. 5, p. 1071–6, 2002.

21 MCGAUGHEY, S. A. et al. Roles of Aquaporins in Setaria viridis Stem Development and Sugar Storage. **Frontiers in Plant Science**, v. 7, p. 1–13, 2016.

22 MCHALE, L. et al. Plant NBS-LRR proteins: adaptable guards. **Genome biology**, v. 7, n. 4, p. 212, 2006.

23 MING, R. et al. The pineapple genome and the evolution of CAM photosynthesis. **Nature Genetics**, v. 47, n. 12, p. 1435–1442, 2015.

24 NAKAMICHI, N. et al. Transcriptional repressor PRR5 directly regulates clock-output pathways. **Proceedings of the National Academy of Sciences**, v. 109, n. 42, p. 17123–17128, 2012.

25 NEKRASOV, V. et al. Control of the pattern-recognition receptor EFR by an ER protein complex in plant immunity. **The EMBO Journal**, v. 28, n. 21, p. 3428–3438, 2009.

26 PADMANABHAN, M. S. et al. Interaction of the Tobacco Mosaic Virus Replicase Protein with the Aux/IAA Protein PAP1/IAA26 Is Associated with Disease Development. **Journal of Virology**, v. 79, n. 4, p. 2549–2558, 2005.

27 PARK, C.-J.; SEO, Y.-S. Heat Shock Proteins: A Review of the Molecular Chaperones for Plant Immunity. **The Plant Pathology Journal**, v. 31, n. 4, p. 323–333, 2015.

28 PENNAZIO, S.; ROGGERO, P. Plant hormones and plant virus diseases. The auxins. **The new microbiologica**, v. 19, n. 4, p. 369–78, 1996.

29 PÉRET, B. et al. Auxin regulates aquaporin function to facilitate lateral root emergence. **Nature Cell Biology**, v. 14, n. 10, p. 991–998, 2012.

30 PERON, F. N. et al. Bioinformatics approach to the study of the molecular behavior of mealybug wilt of pineapple. **Acta Horiculturae**, In press, 2018.

31 PINTO, M. S. T.; RIBEIRO, J. M.; OLIVEIRA, E. A. G . O estudo de genes e proteínas de defesa em plantas. **Revista Brasileira de Biociências**, p. 241–248, 2011.

32 QI, T. et al. The Jasmonate-ZIM-Domain Proteins Interact with the WD-Repeat/bHLH/MYB Complexes to Regulate Jasmonate-Mediated Anthocyanin Accumulation and Trichome Initiation in Arabidopsis thaliana. **The Plant Cell**, v. 23, n. 5, p. 1795–1814, 2011.

33 REDWAN, R. M.; SAIDIN, A.; KUMAR, S. V. The draft genome of MD-2 pineapple using hybrid error correction of long reads. **DNA Research**, v. 23, n. 5, p. 427–439, 2016.

34 SCHOTT, A. et al. Arabidopsis Stromal-derived Factor2 (SDF2) Is a Crucial Target of the Unfolded Protein Response in the Endoplasmic Reticulum. **Journal of Biological Chemistry**, v. 285, n. 23, p. 18113–18121, 2010.

35 SEIFERT, G. J.; XUE, H.; ACET, T. The Arabidopsis thaliana FASCICLIN LIKE ARABINOGALACTAN PROTEIN 4 gene acts synergistically with abscisic acid signalling to control root growth. **Annals of Botany**, v. 114, n. 6, p. 1125–1133, 2014.

36 SETHER, D. M. et al. Diversity and mealybug transmissibility of ampeloviruses in pineapple. **Plant Disease**, v. 89, n. 5, p. 450–456, 2005.

37 SETHER, D. M.; HU, J. S. Closterovirus Infection and Mealybug Exposure Are Necessary for the Development of Mealybug Wilt of Pineapple Disease. **Phytopathology**, v. 92, n. 9, p. 928–935, 2002.

38 SHEN, B. N. et al. Ocurrence and Molecular Characterization of Three Pineapple Mealybug Wilt-Associated Viruses in Pineapple in Taiwan. **Plant Disease**, v. 93, p. 192, 2009.

39 SHIRASU, K.; SCHULZE-LEFERT, P. Regulators of cell death in disease resistance. **Plant molecular biology**, v.44, n. 3, p. 371-385, 2000.

40 SINGH, B.; MAKKAR, H. P. S.; NEGI, S. S. Rate and Extent of Digestion and Potentially Digestible Dry Matter and Cell Wall of Various Tree Leaves. **Journal of Dairy Science**, v. 72, n. 12, p. 3233–3239, 1989.

41 SOUZA, E. H. et al. Genetic variation of the Ananas genus with ornamental potential. **Genetic Resources and Crop Evolution**, v. 59, n. 7, p. 1357–1376, 2012.

42 TOMITA, Y. et al. Mutation of Host dnaJ Homolog Inhibits Brome Mosaic Virus Negative-Strand RNA Synthesis. **Journal of Virology**, v. 77, n. 5, p. 2990–2997, 2003.

43 TRUSOV, Y.; BOTELLA, J. R. Silencing of the ACC synthase gene ACACS2 causes delayed flowering in pineapple [Ananas comosus (L.) Merr.]. **Journal of Experimental Botany**, v. 57, n. 14, p. 3953–3960, 2006.

44 VAN LOON, L. C.; GERAATS, B. P. J.; LINTHORST, H. J. M. Ethylene as a modulator of disease resistance in plants. **Trends in Plant Science**, v. 11, n. 4, p. 184–191, 2006.

45 VENTURA, J. A.; ZAMBOLIM, L. Controle das doenças do abacaxizeiro. In: ZAMBOLIM, L. et al. **Controle de doenças frutíferas**. v.1, Viçosa-MG: UFV, 2002. p. 445–509.

46 WANG, G. et al. RNA-seq analysis of Brachypodium distachyon responses to Barley stripe mosaic virus infection. **The Crop Journal**, v. 5, n. 1, p. 1–10, 2017.

47 WANG, N.; XIAO, B.; XIONG, L. Identification of a cluster of PR4-like genes involved in stress responses in rice. **Journal of Plant Physiology**, v. 168, n. 18, p. 2212–2224, 2011.

48 WHITHAM, S. A. et al. Arabidopsis RTM2 gene is necessary for specific restriction of tobacco etch virus and encodes an unusual small heat shock-like protein. **The Plant cell**, v. 12, n. 4, p. 569–82, 2000.

49 YAMAJI, Y. et al. Lectin-Mediated Resistance Impairs Plant Virus Infection at the Cellular Level. **THE PLANT CELL ONLINE**, v. 24, n. 2, p. 778–793, 2012.

50 ZHANG, J.; LIU, J.; MING, R. Genomic analyses of the CAM plant pineapple. **Journal of Experimental Botany**, v. 65, n. 13, p. 3395–3404, 2014.

51 ZHANG, L.; WANG, A. Virus-induced ER stress and the unfolded protein response. **Frontiers in Plant Science**, v. 3, 2012.

5.3 CAPÍTULO 3

A HIPÓTESE DA RESPOSTA HIPERSENSITIVA AO PMWaV-2

PMWaV-2 é um vírus filamentoso de RNA positivo pertencente a família *Closteroviridae* e gênero *Ampelovirus* (MARTINELLI et al., 2012; SETHER et al., 2009). É detectado em células do floema do abacaxi (SETHER; HU, 2002) podendo ser detectado em plantas sem sintomas evidentes de doenças em plantações com manejo adequado e tratos culturais. Contudo, diversos estudos revelam sua participação no desenvolvimento de sintomas da Murcha do Abacaxizeiro (MWP) (BORROTO-FERNANDEZ; COSTA; LAIMER, 2007; PERON; FERNANDES; VENTURA, 2009; SETHER et al., 2005). Embora se conheça um complexo de PMWaVs, somente o PMWaV-2 é constantemente reportado como o agente determinante da doença. Os dados mostrados nesta Tese corroboram com a literatura e revelam a ocorrência de plantas assintomáticas infectadas por PMWaV-2. Todavia, plantas sintomáticas não apenas apresentam o PMWaV-2 como os níveis de transcritos é significativamente superior ao observado nas plantas assintomáticas da mesma plantação. Assim, pressupõe-se que abacaxizeiros sejam capazes de tolerar a presença do PMWaV-2 até determinado momento no qual ocorre um desbalanço entre os mecanismos alterados pelo vírus e a capacidade de resistência a infecção. A determinação desse momento e mecanismos associados pode conduzir a proposição de alternativas para o controle da MWP. Para tanto, esta Tese contribui com uma hipótese dos mecanismos alterados pela infecção no estágio avançado da doença com base no estudo do sequenciamento do RNA com tecnologia de nova geração. A seguir, os mecanismos alterados são apresentados como parte da construção da hipótese.

1 ESTRESSE OXIDATIVO E OSMÓTICO

A infecção por PMWaV-2 desencadeia uma série de eventos moleculares nas células do floema do abacaxi que resultam na deficiência do transporte de nutrientes e água como promotores do declínio da planta. A doença que ocorre é conhecida por Murcha do abacaxizeiro e apresenta sintomas claros de estresse osmótico e

oxidativo, especialmente nas raízes que, em estágios avançados da doença, morrem. Os mecanismos envolvidos nesta interação envolvem inibição da expressão de genes que codificam diversos transportadores transmembrana como aquaporinas (AQPs) delta-tip, gamma-tip, transportadores de cobre e zinco, além de transportadores de açúcares, lipídios e aminoácidos.

De modo geral, quando as plantas são infectadas por vírus, receptores transmembrana percebem elicitores e desencadeiam o estresse oxidativo com a geração de espécies reativas de oxigênio (EROs) (JOHAL; HULBERT; BRIGGS, 1995; RESENDE, 2010; VLOT; DEMPSEY; KLESSIG, 2009). Por consequinte, uma alteração nos canais de Cálcio também ocorre, aumentando o influxo deste mineral que atua junto aos EROs como mensageiros secundários nas cascatas de sinalização para a expressão de genes de defesa. Então, EROs promove a peroxidação lipídica da membrana celular liberando ácido linolênico que no citoplasma é precursor do ácido jasmônico (AJ). Este hormônio atua sobre fatores de transcrição (FT) que regulam a expressão de genes como os que codificam para proteínas relacionadas à patogênese (PRs). Além de atuar localmente, AJ transloca-se por toda a planta sinalizando para a ativação prévia das defesas celulares de forma sistêmica (PINTO; RIBEIRO; OLIVEIRA, 2011; STRATMANN, 2003). Essa estratégia objetiva fortalecer paredes celulares e conter o patógeno no sítio de infecção.

Em se tratando da infecção por PMWaV-2 em abacaxizeiros, os genes que codificam para a expressão de enzimas determinantes na formação do AJ como AOS e LOX5, são reprimidas inviabilizando o acúmulo deste importante sinalizador de estresse. Além disso, fatores de transcrição envolvidos na regulação da expressão de genes de defesa e responsivos ao AJ também são reprimidos, como o WRKY33, WRKY40 e WRKY51. Portanto, essa modulação inibe a expressão de PRs relacionadas a resposta sistêmica adquirida (SAR) e outras proteínas prejudicando o mecanismo de defesa. As PRs são de extrema importância para manter o patógeno no local da infecção e na presença do PMWaV-2, *PR5* (osmotina), *PR4*, *CHIA* (quitinase) e *PDCB3* (glucanase) são inibidas aumentando a suscetibilidade a infecções decorrente da perturbação oxidativa e osmótica da membrana celular e da parede celular. Como o acúmulo de EROs é citotóxico,

enzimas removedoras como as superóxidos dismutase (SODs), lacases, peroxidases e FLAs, são fundamentais para a manutenção da integridade da parede celular e da homeostase. Na presença do PMWaV-2, os genes que codificam para essas enzimas são inibidos conferindo um intenso estresse oxidativo e osmótico (Figura 1).

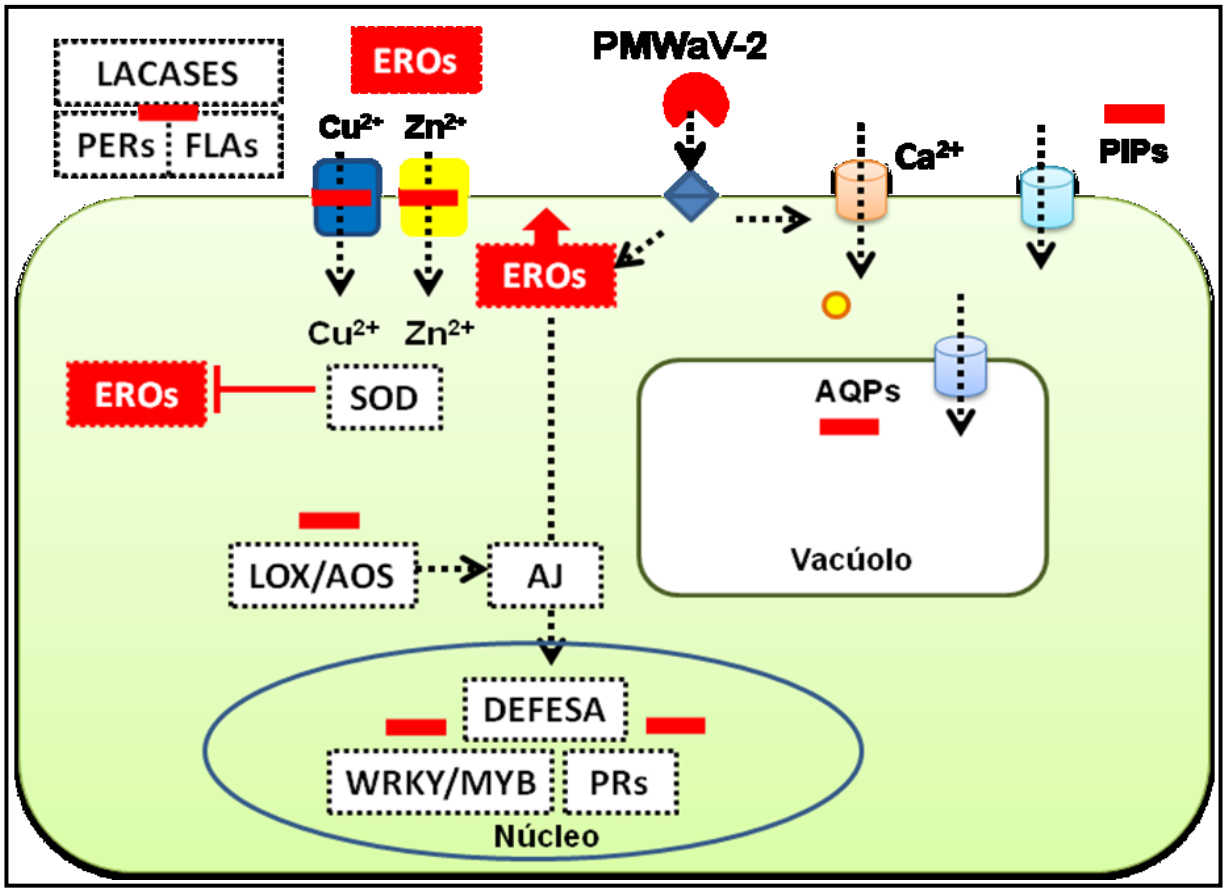


Figura 1 Esquema representativo do estresse oxidativo e osmótico induzido por PMWaV-2 em células do floema de abacaxi com sintomas de murcha. Os genes com expressão reprimida estão representados com sinal negativo em vermelho.

Além dos transportadores de Cobre e Zinco que são essencias para as SODs, diversas AQPs são reprimidas contribuindo para o sintoma clássico da doença que é a perda de turgidez.

2 CROSS-TALK HORMONAL E O DESENVOLVIMENTO DE SINTOMAS

Se por um lado as defesas vegetais mediadas por AJ são reprimidas (Figura 1 e 2), a regulação por auxina e etileno (ET) são induzidas (Figura 2).

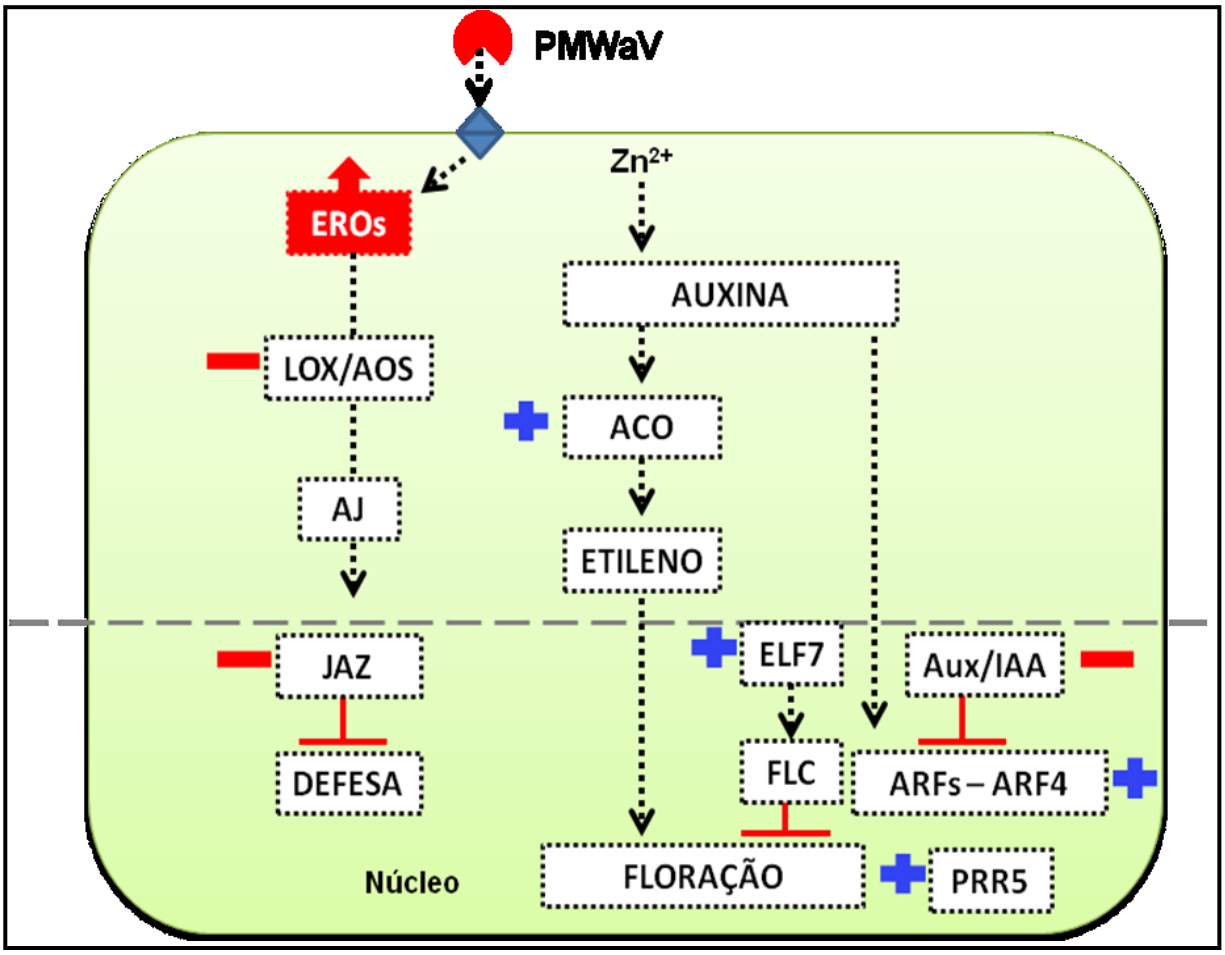


Figura 2 Representação dos genes envolvidos no *cross-talk* hormonal em plantas de abacaxi com sintomas de murcha. Genes com expressão reprimida e induzida estão representados por sinais negativo vermelho e positivo azul, respectivamente.

A produção de ET é auxiliada pela participação da auxina na conversão de S-adenosil-L-metionina (SAM) a enzima ACO que é determinante na fase final de formação do ET (XU; ZHANG, 2015). No abacaxi, a transição da fase vegetativa para a reprodutiva é irreversível e iniciada pela formação do etileno (REDWAN; SAIDIN; KUMAR, 2016; TRUSOV; BOTELLA, 2006). Como *ACO* é positivamente regulado em plantas com murcha e estas dificilmente chegam a frutificar, propõe-se que ocorra regulação da floração pela relação de auxina e etileno. Ademais outros genes reguladores da floração induzidos no abacaxi reforçam esta hipótese. *PRR5,*

por exemplo, participa do controle do ritmo circadiano em *Arabidospsis* e sua indução é associada a antecipação da floração (ZHANG; LIU; MING, 2014). Por outro lado, a indução da expressão de *ELF7* aponta para a modulação uma vez que em *Arabidopsis* é requerido para ativar FLC e atuar no bloqueio da expressão de genes envolvidos na floração (Figura 2) (HE; DOYLE; AMASINO, 2004).

A auxina não apenas participa indiretamente da sinalização por ET como ativa a expressão de genes associados ao desenvolvimento de sintomas como a distorção foliar (PENNAZIO; ROGGERO, 1996; PÉRET et al., 2012) através de fatores de transcrição ARF. ARF4, por exemplo, é expresso em situações de estresse hídrico e atua na regulação da abertura estomática em plantas de metabolismo CAM (ABRAHAM et al., 2016). Por outro lado, a inibição de FTs Aux/IAA podem estar associadas à contenção do vírus PMWaV-2 no local da infecção além de desencadear sintomas de murcha. Isso porque em *Arabidospsis* já foi demonstrada a associação de um FT Aux/IAA, a proteína PAP1, com o *Tobacco mosaic vírus* (TMV) com consequente perda da dominância apical (PADMANABHAN et al., 2005). Diante disso, a regulação da auxina é sugerida como mecanismo de defesa (MAYDA et al., 2000) enquanto que o *cross-talk* com o etileno é assocaido a sintomas como epinastia. Portanto, a sinalização por jasmonato, auxina e etileno em plantas de abacaxi com sintomas de murcha revelam parte do mecanismo envolvido no desenvolvimento de sintomas. Nesse processo, a via SAR é suprimida em vários pontos da sinalização a começar pela biossíntese do jasmonato, inibição de FTs responsivos a AJ e inibição de PRs. Concomitantemente, a auxina disponível contribui com a sinalização do etileno para a floração e ativa a expressão de genes que participam da resposta ao estresse hídrico. A inibição de genes envolvidos nesses mecanismos interfere no desenvolvimento da planta e contribuem para o aparecimento de sintomas.

3 RESISTÊNCIA MEDIADA POR GENES DE RESISTÊNCIA

O sucesso da infecção do PMWaV-2 no abacaxi depende da capacidade em se deslocar para outras células. Por pertencer a família *Closteroviridae* (MARTINELLI et al., 2012), acredita-se que esse vírus se desloque de célula a célula via

plasmodesmo (PCD) e sistemicamente via fluxo do floema. Da mesma forma, *Tobacco etch vírus* (TEV) se movimenta em *Arabidopsis* tendo sido demonstrada sua restrição mediada pela chaperona hsp20 RTM2 (WHITHAM et al., 2000). Em plantas de abacaxi infectadas por PMWaV-2 observa-se uma correlação negativa entre os níveis de transcritos do vírus e de *RTM2*. Além de revelar uma provável interação, a observação dos sintomas em plantas que apresentam maior nível de transcritos virais demonstra a necessidade da expressão de *RTM2* no desenvolvimento de resistência ao PMWaV-2.

RTM2 é um gene do tipo *R*, da classe *RTM* (*Restricted Tobacco etch vírus Movement*), que é expresso em resposta ao gene de avirulência (*Avr)* do vírus para o desenvolvimento de resistência e que codifica para uma proteína de choque térmico hsp20. Proteína desta natureza foi detectada por espectrometria de massas em plantas de abacaxi assintomáticas contendo menor nível de transcritos de PMWaV-2 não tendo sido identificada em plantas com sintomas severos da doença. Esse dado fortalece a hipótese do envolvido de hsp20 no mecanismo de resistência do abacaxi ao PMWaV-2 (Figura 3).

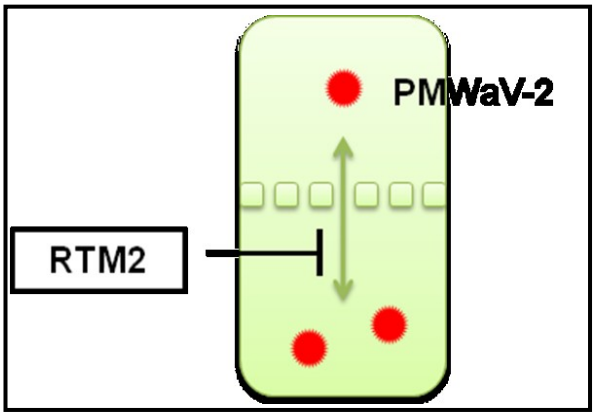


Figura 3 Ilustração da hipótese do modo de ação da hsp20 RTM2 sobre o deslocamento do PMWaV-2 via floema para longas distâncias. A expressão de RTM2 pode restringir o movimento das partículas virais limitando sua disseminação e multiplicação.

Nesse processo de determinação da resistência outros genes *R* podem ser expressos como os pertencentes a maior classe, os NBS-LRR. Na presença do PMWaV-2, a expressão de Aco016970, que codifica para uma proteína de resistência a doença da classe NBS-LRR e transportadora de cobre, também é

modulada. O abacaxi, embora suscetível ao PMWaV-2, apresenta genes de resistência das classes NBS-LRR e RTM que são diferencialmente expressos em razão da infecção e que podem atuar ativamente no desenvolvimento de resistência.

4 RESISTÊNCIA MEDIADA POR CHAPERONAS

Os vírus são parasitas obrigatórios e, portanto, apresentam mecanismos para contornar as defesas da planta sem que a mesma venha a óbito. Contudo, quando as plantas não conseguem conter a multiplicação e disseminação do vírus, e nem manter a homeostase celular, a apoptose é ativada e o declínio da planta é decorrente. Em situações de estresse na membrana plasmática, como o causado pela infecção por vírus, além do estresse oxidativo ocorre aumento do influxo de cálcio no citoplasma. Este mineral atua como mensageiro secundário na sinalização do estresse sendo carreado por calmodulina (CaM) e estocado no RE. No lúmen do retículo, o cálcio é sequestrado por calreticulina (CRT) que atua como chaperonina no dobramento de proteínas ou marcação para a degradação. Sua associação a proteínas de movimento do vírus do mosaico TMV é relatada como auxiliar no deslocamento do vírus via plasmodesmo (PCD) (CHEN et al., 2005).

O dobramento das proteínas secretoras do RE, incluindo os transportadores transmembrana, é dependente das chaperonas. Nesse processo uma proteína de choque térmico HSP70 dependente de cálcio, BiP, é a principal chaperona que funciona como elemento chave no controle da qualdiade do RE. Em condições homeostáticas, BiP permanece associada a proteínas trasmembranas como a IRE1 (HÜTTNER; STRASSER, 2012; MAULE; LEH; LEDERER, 2002; PARK; SEO, 2015; ZHANG; WANG, 2012). No lúmen, proteínas mal dobradas são ubiquitindas com o auxílio de chaperonas como a ERDJ3B, e CRT e posteriormente são direcionadas para a via da degradação associada ao RE, ERAD (do inglês, *endoplasmic-reticulum-associated protein degradation*), no citoplasma. Por outro lado, proteínas não dobradas são identificadas pela associação de SDF2 com ERDJ3B para o dobramento realizado junto a BiP. Com o aumento da atividade traducional, a liberação de BiP no lúmen do RE desencadeia sinalização para a expressão de mais chaperonas e elementos da via ERAD através da via de resposta a proteína não

dobrada UPR (do inglês, *unfolded protein response*) (NEKRASOV et al., 2009). Esta via objetiva controlar a qualidade do funcionamento da maquinaria traducional mantendo a homoeostase celular. Quando a pressão por mais chaperonas é constante e a demanda se torna insustentável, a dissociação entre BiP e IRE1 sinaliza UPR para a MCP por apoptose (Figura 4) (HÜTTNER; STRASSER, 2012; MAULE; LEH; LEDERER, 2002; PARK; SEO, 2015; ZHANG; WANG, 2012).

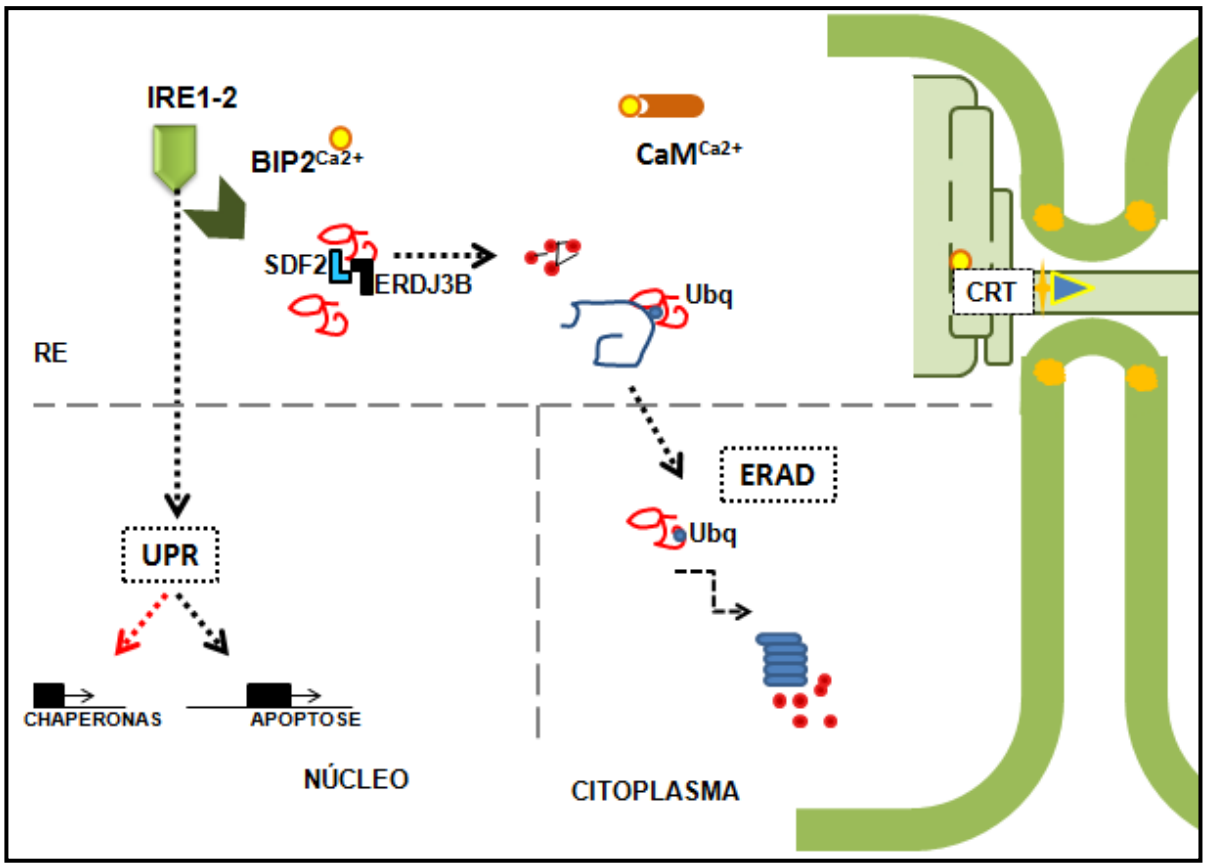


Figura 4 Modelo de ativação da via UPR mediada por sinalização da proteína IRE1-2 a partir do retículo endoplasmático (RE) na célula de abacaxi. O dobramento de proteínas no interior do RE é auxiliado por chaperonas como ERDJ3B e BiP2. Nesse processo, com o auxílio de SDF2, a ERDJ3B identifica proteínas não dobradas para que BiP2 possa encaixar e conduzir o dobramento correto da proteína. Aquelas mal dobradas são ubiquitinadas e direcionadas para a degradação na via ERAD no citoplasma. A via de resposta à proteína não dobrada (UPR) é ativada para induzir a expressão de mais chaperonas e restabelecer o dobramento das proteínas geradas. O excesso dessas proteínas promove estresse no RE e redireciona a UPR para a ativação da morte celular programada por apoptose.

Na infecção do abacaxi por PMWaV-2 ocorre ativação da UPR, contudo, em plantas sintomáticas com elevado nível de transcritos virais, a expressão de *BiP2*, *ERDJ3B*, *SDF2*, *CaM*, *CRT* e Ubiquitina (*Ubq*) são inibidas sendo detectados somente em plantas assintomáticas com menor nível de transcritos virais. Ademais, a expressão de *IRE1-2* é positivamente regulada nas plantas com sintomas severos da murcha o que fundamenta a hipótese da ativação da apoptose no estágio avançado da doença como última medida de contenção da infecção local.

A inibição da expressão de chaperonas DNAJ e hsp70 como estratégia de contenção da replicação de vírus da família *Closteroviridae* também é relatada por Tomita *et al*., 2003 e Espinoza *et al*., 2007. Portanto, os dados apresentados suportam a hipótese da inibição de chaperonas no abacaxi como mecanismo de defesa contra o PMWaV-2.

Em interações incompatíveis como a que ocorre entre o abacaxi e o PMWaV-2, o RE sinaliza para uma resposta hipersensitiva (HR) que tem por característica induzir a expressão de PRs acompanhado do estresse oxidativo e promover a MCP (YE *et al*., 2011; XU *et al*., 2012). A própria expressão de *PRs*, como *PR1* a exemplo, é dependente da expressão de BiP que intervém no dobramento de NPR1 para que ocorra a ativação da expressão de *PR1* (WANG, 2005). Assim, a inibição da expressão de *PRs* em plantas sintomáticas de abacaxi pode estar associada a redução da expressão de *BiP*, o que explica a dificuladade em conter o vírus e revela o provável mecanismo de ativação da morte celular no estágio avançado da murcha. Além disso, a glucanase PDCB3 que é expressa como HR favorece o fluxo de partículas virais de célula a célula. A inibição da expressão desta *PR* no abacaxi caracteriza uma estratégia da planta de conter o vírus no local de infecção contribuindo para o isolamento da célula (Figura 5).

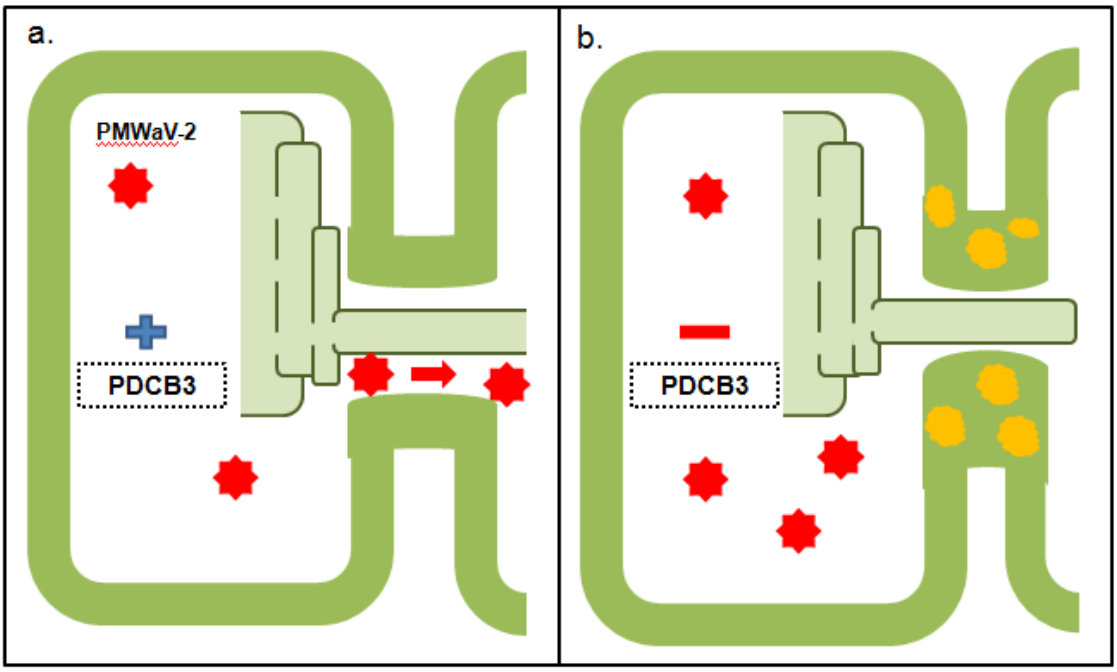


Figura 5 Ilustração do efeito da glucanase PDCB3 no movimento de célula a célula do PMWaV-2 em células de abacaxi. Na presença da glucanase (a), a deposição de calose é controlada favorecendo o deslocamento de partículas virais via PD. Na ausência da glucanase (b) e presença de PMWaV-2, a HR induz a deposição de calose (representada em amarelo) que aumenta a densidade da parede celular limitando o fluxo de partículas no PD.

Diante do exposto, pode-se afirmar que a infecção por PMWaV-2 causa estresse no RE ativando a HR através da via UPR. Nesse processo, chaperonas são inibidas visando conter a replicação e o deslocamento viral ativando a apoptose.

5 HIPÓTESE

PMWaV-2 causa estresse oxidativo, osmótico e no RE. Inibe as defesas vegetais a nível de via SAR mediada por AJ e genes de resistência das classes NBS-LRR e RTM, com destaque para *RTM2* que codifica uma chaperona hsp20. Para conter o vírus no local da infecção, o abacaxi inibe a expressão de potenciais facilitadores da replicação e dispersão do PMWaV-2, as chaperonas BiP2 e ERDJ3B, a chaperonina CRT, CaM, FTs Aux/IAA e a glucanase PDCB3. A resultante da relação entre os mecanismos alterados pelo vírus e as estratégias moleculares de defesa da planta constituem uma resposta hipersensitiva com ativação da apoptose.

6 CONCLUSÕES

Este é o primeiro estudo que avalia o transcriptoma de abacaxizeiro infectado por PMWaV-1 e PMWaV-2 usando RNA-seq, o mapeador STAR e o genoma de referência *A.comosus* v3, tendo sido indentificados 16.097 genes expressos.

A análise comparativa do transcriptoma de plantas sintomáticas e assintomáticas usando o software DESeq2 revelou 390 DEGs sendo 122 induzidos e 268 reprimidos em plantas sintomáticas. O conjunto DEGs identificado por EdgeR foi também identificado em 93% por DESeq2.

Sintomas severos de Murcha do abacaxizeiro são percebidos em plantas infectadas por PMWaV-2 e embora este vírus possa ser detectado em plantas assintomáticas, sua expressão é significativamente maior em plantas sintomáticas.

O estudo do transcriptoma de plantas de abacaxi infectadas por PMWaV-2 assintomáticas que coexistem no campo ao lado de plantas sintomáticas revelam mecanismos de tolerância suprimidos que podem ser alvo de melhoramento das defesas do abacaxi.

As análises funcionais dos DEGs revelaram que o estresse causado por PMWaV-2 induziu alterações na homeostase da célula reprimindo a expressão de diversas aquaporinas e transportadores de Cobre e Zinco perturbando o equilíbrio osmótico e a manutenção da parede celular.

A infecção por PMWaV-2 regulou negativamente SAR, UPR, ERAD e genes *R*. A supressão dessas defesas da planta levou ao desenvolvimento da doença e envolveu uma regulação hormonal mediada por Auxina, Etileno e Jasmonato.

A predição de interação proteína-proteína revelou BiP como uma chaperona chave na regulação das respostas ao PMWaV. A identificação das proteínas HSP70, CaM e CRT nas plantas assintomáticas fundamentam a hipótese de que o vírus causa estresse no retículo endoplasmático suprimindo demais defesas e direcionando UPR para apoptose.

A identificação de uma correlação negativa entre a expressão gênica de *RTM2* e PMWaV-2 e da expressão de proteína HSP20 em plantas assintomáticas, indicam o gene *RTM2* como forte candidato ao estudo de resistência.

Com a exposição prolongada ao PMWaV-2, plantas sintomáticas reprimem a expressão das chaperonas *DNAJs*, *HSP70* e *CRT* na provável tentativa de contenção do vírus no sítio de infecção. Da mesma forma reprimem a expressão de *PDCB3* que interfere no controle da calose e deslocamento do vírus no plasmodesmo.

Os genes induzidos em decorrência da doença estão associados a respostas a Auxina e regulação da floração. Supõe-se que proteínas virais alterem as respostas à auxina aumentando sua virulência e que a planta antecipe a floração como estratégia evolutiva para a conclusão do ciclo de vida. Nesse contexto, sintomas de murcha como a epinastia podem estar relacionados com a modulação das respostas à auxina.

Os sintomas de murcha decorrem do estresse osmótico, estresse oxidativo, deficiente sinalização de cálcio e estresse no retículo endoplasmático. Assim, esta Tese fundamenta a necessidade da adequação do manejo da doença murcha do abacaxizeiro e do cultivo do abacaxi com proposição de suplementação de nutrientes como o Cobre.

Adicionalmente, propõe-se o tratamento da cultura do abacaxi com aplicação de Jasmonato em período que antecede a indução da floração por Etileno, para a indução de resistência e fortalecimento da parede celular. Essa estratégia objetiva promover defesas vegetais contra as duas principais doenças que prejudicam a abacaxizultura mundial, a Fusariose e a Murcha do Abacaxizeiro.

Assim, este trabalho fornece diretrizes para estudos de melhoramento de plantas com atenção especial ao gene *RTM2* como um possível regulador da resistência ao PMWaV e a via UPR como mediadora dos mecanismos de defesa.

7 REFERÊNCIAS

1 ABRAHAM, P. E. et al. Transcript, protein and metabolite temporal dynamics in the CAM plant Agave. **Nature Plants**, v. 2, n. 12, p. 16178, 2016.

2 AGRANOVSKY, A. A. Closteroviruses: Molecular Biology, Evolution and Interactions with Cells. In: **Plant Viruses: Evolution and Management**. Singapore: Springer Singapore, p. 231–252, 2016.

3 ALBRECHT, U.; BOWMAN, K. D. Gene expression in Citrus sinensis (L.) Osbeck following infection with the bacterial pathogen `Candidatus Liberibacter asiaticus' causing Huanglongbing in Florida. **Plant Sci**, v. 175, 2008.

4 BORROTO-FERNÁNDEZ, E. G.; TORRES-ACOSTA, J. A.; LAIMER, M. RT-PCR detection and protein-protein interaction of viral components of pineapple mealybug wilt-associated virus 2 in Cuba. **Journal of Plant Pathology**, v. 89, p. 435–439, 2007.

5 CARTER, W. Ecological Aspects of Plant Virus Transmissions. **Annual Review of Entomology**, v. 6, n. 1, p. 347–370, 1961.

6 CARTER, W. Injuries to Plants Caused by Insect Toxins. **Botanical Review**, v. 5, p. 273–326, 1939.

7 CHEN, C. et al. Transcriptome Profiling of the Pineapple under Low Temperature to Facilitate Its Breeding for Cold Tolerance. **PLOS ONE**, v. 11, n. 9, p. e0163315, 2016.

8 CHISHOLM, S. T. et al. Arabidopsis RTM1 and RTM2 Genes Function in Phloem to Restrict Long-Distance Movement of Tobacco Etch Virus. **PLANT PHYSIOLOGY**, v. 127, n. 4, p. 1667–1675, 2001.

9 CHITWOOD, D. H.; TIMMERMANS, M. C. P. Small RNAs are on the move. **Nature**, v. 467, n. 7314, p. 415–419, 2010.

10 COSTA, H. B. et al. A new procedure based on column chromatography to purify bromelain by ion exchange plus gel filtration chromatographies. **Industrial Crops and Products**, v. 59, p. 163–168, 2014.

11 DEY, K. K. et al. Analysis of pineapple mealybug wilt associated virus -1 and -2 for potential RNA silencing suppressors and pathogenicity factors. **Viruses**, v. 7, n. 3, p. 969–995, 2015.

12 DING, S.-W. RNA-based antiviral immunity. **Nature Reviews Immunology**, v. 10, n. 9, p. 632–644, 2010.

13 FAO. **Food and Agriculturae Organization of the United Nations - FAOSTAT**. Disponível em: <http://www.fao.org/faostat/em/#home>. Acesso em: 01 jun. 2018.

14 GAMBLEY, C. F. et al. The genetic diversity of ampeloviruses in Australian pineapples and their association with mealybug wilt disease. **Australasian Plant Pathology**, v. 37, n. 2, p. 95–105, 2008.

15 GAO, Q.-M. et al. Signal regulators of systemic acquired resistance. **Frontiers in Plant Science**, v. 06, 2015.

16 GLAZEBROOK, J. Contrasting Mechanisms of Defense Against Biotrophic and Necrotrophic Pathogens. **Annual Review of Phytopathology**, v. 43, n. 1, p. 205–227, 2005.

17 GUNASINGHE, U. B. Purification and Partial Characterization of a Virus from Pineapple. **Phytopathology**, v. 79, n. 12, p. 1337, 1989.

18 HAO, Y. et al. Transcriptome Analysis Provides Insights into the Mechanisms Underlying Wheat Plant Resistance to Stripe Rust at the Adult Plant Stage. **PLOS ONE**, v. 11, n. 3, p. e0150717, 2016.

19 HE, Y.; DOYLE, M. R.; AMASINO, R. M. PAF1-complex-mediated histone methylation of FLOWERING LOCUS C chromatin is required for the vernalization-responsive, winter-annual habit in Arabidopsis. **Genes & Development**, v. 18, n. 22, p. 2774–2784, 2004.

20 HÜTTNER, S.; STRASSER, R. Endoplasmic Reticulum-Associated Degradation of Glycoproteins in Plants. **Frontiers in Plant Science**, v. 3, 2012.

21 JAIN, M. Next-generation sequencing technologies for gene expression profiling in plants. **Briefings in Functional Genomics**, v. 11, n. 1, p. 63–70, 2012.

22 JOHAL, G. S.; HULBERT, S. H.; BRIGGS, S. P. Disease lesion mimics of maize: A model for cell death in plants. **BioEssays**, v. 17, n. 8, p. 685–692, 1995.

23 KAMITANI, M. et al. RNA-Seq reveals virus–virus and virus–plant interactions in nature. **FEMS Microbiology Ecology**, v. 92, n. 11, p. fiw176, 2016.

24 LIU, C.; FAN, C. De novo Transcriptome Assembly of Floral Buds of Pineapple and Identification of Differentially Expressed Genes in Response to Ethephon Induction. **Frontiers in Plant Science**, v. 7, p. 203, 2016.

25 LOPES, K. J. A. **Desenvolvimento de um processo de limpeza clonal de abacaxizeiro para Pineapple mealybug wilt associated virus (PMWaV) com o uso da hidrotermoterapia**. Dissertação (Mestrado em Biotencologia) - Programa de Pós-Graduação em Biotecnologia, Universidade Federal do Espírito Santo. Espírito Santo, 57f. 2018.

26 MANICA, I. **Abacaxi: do plantio ao mercado**. 1. ed. Porto Alegre: Ed. Cinco Continentes, 2000. 122p.

27 MARTIN, J. A.; WANG, Z. Next-generation transcriptome assembly. **Nature Reviews Genetics**, v. 12, n. 10, p. 671–682, 2011.

28 MARTINELLI, G. P. et al. Taxonomic Revision of the family Closteroviridae with special reference to the Grapevine leafroll-associated members if the genus Ampelovirus and the putative species unassigned to the family. **Jornal of Plant Pathology**, v. 94, n. 1, p. 7–19, 2012.

29 MAULE, A.; LEH, V.; LEDERER, C. The dialogue between viruses and hosts in compatible interactions. **Current Opinion in Plant Biology**, v. 5, n. 4, p. 279–284, 2002.

30 MAYDA, E. et al. Expression of a Pathogen-Induced Gene Can Be Mimicked by Auxin Insensitivity. **Molecular Plant-Microbe Interactions**, v. 13, n. 1, p. 23–31, 2000.

31 MAYO, M. A. Virus taxonomy. **Archives of virology**, v. 147, n. 5, p. 1071–6, 2002.

32 MCHALE, L. et al. Plant NBS-LRR proteins: adaptable guards. **Genome biology**, v. 7, n. 4, p. 212, 2006.

33 MELZER, M. J. et al. Complete nucleotide sequence and genome organization of pineapple mealybug wilt-associated virus-1. **Archives of Virology**, v. 153, n. 4, p. 707–714, 2008.

34 MELZER, M. J. et al. Nucleotide sequence, genome organization and phylogenetic analysis of pineapple mealybug wilt-associated virus-2. **Archives of Virology**, v. 153, n. 4, p. 707–714, 2001.

35 MING, R. et al. The pineapple genome and the evolution of CAM photosynthesis. **Nature Genetics**, v. 47, n. 12, p. 1435–1442, 2015.

36 MOROZOVA, O.; HIRST, M.; MARRA, M. A. Applications of New Sequencing Technologies for Transcriptome Analysis. **Annual Review of Genomics and Human Genetics**, v. 10, n. 1, p. 135–151, 2009.

37 MUR, L. A. J. The Outcomes of Concentration-Specific Interactions between Salicylate and Jasmonate Signaling Include Synergy, Antagonism, and Oxidative Stress Leading to Cell Death. **PLANT PHYSIOLOGY**, v. 140, n. 1, p. 249–262, 2005.

38 NEKRASOV, V. et al. Control of the pattern-recognition receptor EFR by an ER protein complex in plant immunity. **The EMBO Journal**, v. 28, n. 21, p. 3428–3438, 2009.

39 ONG, W. D.; VOO, L. C.; KUMAR, V. S. De Novo Assembly , Characterization and Functional Annotation of Pineapple Fruit Transcriptome through Massively Parallel Sequencing. **PLoS ONE**, v. 7, n. 10, p. e46937, 2012.

40 PADMANABHAN, M. S. et al. Interaction of the Tobacco Mosaic Virus Replicase Protein with the Aux/IAA Protein PAP1/IAA26 Is Associated with Disease Development. **Journal of Virology**, v. 79, n. 4, p. 2549–2558, 2005.

41 PARK, C.-J.; SEO, Y.-S. Heat Shock Proteins: A Review of the Molecular Chaperones for Plant Immunity. **The Plant Pathology Journal**, v. 31, n. 4, p. 323–333, 2015.

42 PENNAZIO, S.; ROGGERO, P. Plant hormones and plant virus diseases. The auxins. **The new microbiologica**, v. 19, n. 4, p. 369–78, 1996.

43 PÉRET, B. et al. Auxin regulates aquaporin function to facilitate lateral root emergence. **Nature Cell Biology**, v. 14, n. 10, p. 991–998, 2012.

44 PEREZ, P. E. et al. Characterization and control of pineapple mealybug wilt associated ampeloviruses. **Acta Horticulturae**, n. 702, p. 23-27, 2006.

45 PERON, F. N.; FERNANDES, P. M. B.; VENTURA, J. A. Detecção de PMWaV-1 e PMWaV-2 em abacaxizeiros no Estado do Espírito Santo. **Tropical Plant Pathology**, v. 34, p. 268, 2009.

46 PERVIEUX, I. et al. A spruce defensin showing strong antifungal activity and increased transcript accumulation after wounding and jasmonate treatments. **Physiological and Molecular Plant Pathology**, v. 64, n. 6, p. 331–341, 2004.

47 PINTO, M. S. T.; RIBEIRO, J. M.; OLIVEIRA, E. A. G . O estudo de genes e proteínas de defesa em plantas. **Revista Brasileira de Biociências**, p. 241–248, 2011.

48 PY, C.; LACOEUILHE, J. J.; TEISSON, C. L'ananas : sa culture, ses produits. In: **Techniques agricoles et productions tropicales**. Paris: Maisonneuve & Larose, and Agence de Coopération Culturelle et Technique, 1984, n. 33, p.499-532.

49 QI, T. et al. The Jasmonate-ZIM-Domain Proteins Interact with the WD-Repeat/bHLH/MYB Complexes to Regulate Jasmonate-Mediated Anthocyanin Accumulation and Trichome Initiation in Arabidopsis thaliana. **The Plant Cell**, v. 23, n. 5, p. 1795–1814, 2011.

50 RAMLI, A. N. M.; AZNAN, T. N. T.; ILLIAS, R. M. Bromelain: from production to commercialisation. **Journal of the Science of Food and Agriculture**, v. 97, n. 5, p. 1386–1395, 2017.

51 REDWAN, R. M.; SAIDIN, A.; KUMAR, S. V. The draft genome of MD-2 pineapple using hybrid error correction of long reads. **DNA Research**, v. 23, n. 5, p. 427–439, 2016.

52 RESENDE, M. L. V. ET AL. O papel dos MAMPs na imunidade inata em plantas. **Revisão Anual de Patologia de Plantas**, v. 18, p. 1–50, 2010.

53 REYMOND, P. Differential Gene Expression in Response to Mechanical Wounding and Insect Feeding in Arabidopsis. **THE PLANT CELL ONLINE**, v. 12, n. 5, p. 707–720, 2000.

54 SANCHES, N. F. Manejo integrado da cochonilha do abacaxi. **Embrapa Mandioca e Fruticultura Tropical**. Abacaxi em Foco, 35, 2005.

55 SETHER, D. M. et al. Differentiation, Distribution, and Elimination of Two Different Pineapple mealybug wilt-associated viruses Found in Pineapple. **Plant Disease**, v. 85, n. 8, p. 856–864, 2001.

56 SETHER, D. M. et al. Diversity and mealybug transmissibility of ampeloviruses in pineapple. **Plant Disease**, v. 89, n. 5, p. 450–456, 2005.

57 SETHER, D. M. et al. Genome organization and phylogenetic relationship of Pineapple mealybug wilt associated virus-3 with family Closteroviridae members. **Virus Genes**, v. 38, n. 3, p. 414–420, 2009.

58 SETHER, D. M.; HU, J. S. Closterovirus Infection and Mealybug Exposure Are Necessary for the Development of Mealybug Wilt of Pineapple Disease. **Phytopathology**, v. 92, n. 9, p. 928–935, 2002.

59 SETHER, D. M.; ULLMAN, D. E.; HU, J. S. Transmission of Pineapple Mealybug Wilt-Associated Virus by Two Species of Mealybug (Dysmicoccus spp.). **Phytopathology**, v. 88, n. 11, p. 1224–1230,1998.

60 SHEN, B. N. et al. Ocurrence and Molecular Characterization of Three Pineapple Mealybug Wilt-Associated Viruses in Pineapple in Taiwan. **Plant Disease**, v. 93, p. 192, 2009.

61 SIVASANKAR, S.; SHELDRICK, B.; ROTHSTEIN, S. J. Expression of allene oxide synthase determines defense gene activation in tomato. **Plant physiology**, v. 122, n. 4, p. 1335–42, 2000.

62 STRATMANN, J. Long distance run in the wound response – jasmonic acid is pulling ahead. **Trends in Plant Science**, v. 8, n. 6, p. 247–250, 2003.

63 SUBERE, C. V. Q. et al. DETECTION AND ABSOLUTE QUANTIFICATION OF PINEAPPLE MEALYBUG WILT-ASSOCIATED VIRUS-2 IN PINEAPPLE USING REAL-TIME RT-PCR (TAQMAN®) ASSAYS. **Acta Horticulturae**, n. 902, p. 349–355, 2011.

64 TRUMAN, W. et al. Arabidopsis systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. **Proceedings of the National Academy of Sciences**, v. 104, n. 3, p. 1075–1080, 2007.

65 TRUMAN, W. M. et al. Arabidopsis Auxin Mutants Are Compromised in Systemic Acquired Resistance and Exhibit Aberrant Accumulation of Various Indolic Compounds. **PLANT PHYSIOLOGY,** v. 152, n. 3, p. 1562–1573, 2010.

66 TRUSOV, Y.; BOTELLA, J. R. Silencing of the ACC synthase gene ACACS2 causes delayed flowering in pineapple [Ananas comosus (L.) Merr.]. **Journal of Experimental Botany**, v. 57, n. 14, p. 3953–3960, 2006.

67 VAN LOON, L. C.; GERAATS, B. P. J.; LINTHORST, H. J. M. Ethylene as a modulator of disease resistance in plants. **Trends in Plant Science**, v. 11, n. 4, p. 184–191, 2006.

68 VENTURA, J. A.; ZAMBOLIM, L. Controle das doenças do abacaxizeiro. In: ZAMBOLIM, L. et al. **Controle de doenças frutíferas**. v.1, Viçosa-MG: UFV, 2002. p. 445–509.

69 VIRALZONE. **Ampelovirus**. Disponível em: <http://viralzone.expasy.org/all_by_species/285.html>. Acesso em: 01 jun. 2018.

70 VLOT, A. C.; DEMPSEY, D. A.; KLESSIG, D. F. Salicylic Acid, a Multifaceted Hormone to Combat Disease. **Annual Review of Phytopathology**, v. 47, n. 1, p. 177–206, 2009.

71 WANG, C. et al. Free Radicals Mediate Systemic Acquired Resistance. **Cell Reports**, v. 7, n. 2, p. 348–355, 2014.

72 WANG, D. Induction of Protein Secretory Pathway Is Required for Systemic Acquired Resistance. **Science**, v. 308, n. 5724, p. 1036–1040, 2005.

73 WANG, G. et al. RNA-seq analysis of Brachypodium distachyon responses to Barley stripe mosaic virus infection. **The Crop Journal**, v. 5, n. 1, p. 1–10, 2017.

74 WHITHAM, S. A. et al. Arabidopsis RTM2 gene is necessary for specific restriction of tobacco etch virus and encodes an unusual small heat shock-like protein. **The Plant cell**, v. 12, n. 4, p. 569–82, 2000.

75 XU, J.; ZHANG, S. Ethylene Biosynthesis and Regulation in Plants. In: **Ethylene in Plants**. Dordrecht: Springer Netherlands, 2015. p. 1–25.

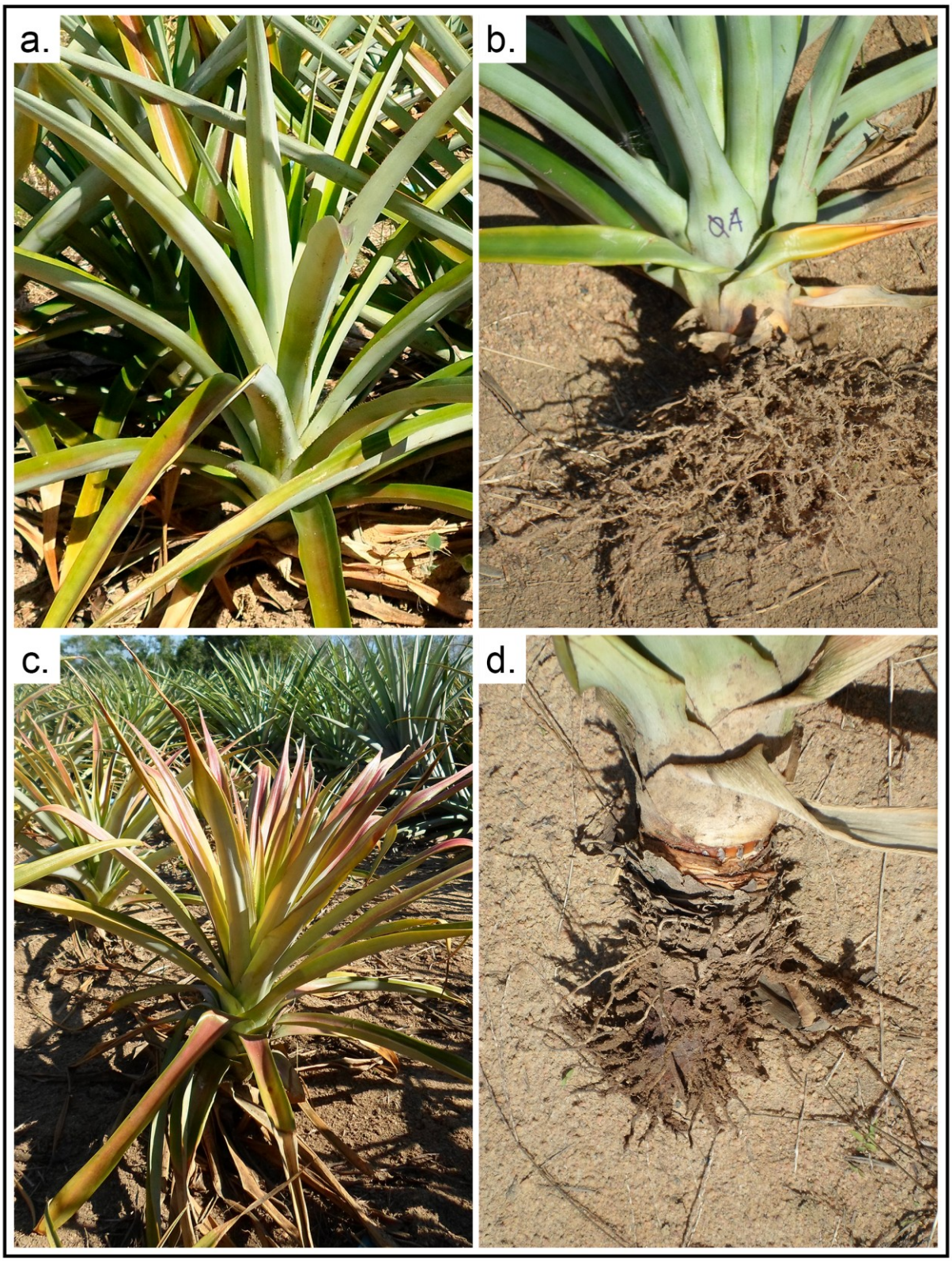
76 YAMAJI, Y. et al. Lectin-Mediated Resistance Impairs Plant Virus Infection at the Cellular Level. **THE PLANT CELL ONLINE**, v. 24, n. 2, p. 778–793, 2012.

77 ZHANG, J.; LIU, J.; MING, R. Genomic analyses of the CAM plant pineapple. **Journal of Experimental Botany**, v. 65, n. 13, p. 3395–3404, 2014.

78 ZHANG, L.; WANG, A. Virus-induced ER stress and the unfolded protein response. **Frontiers in Plant Science**, v. 3, 2012.

79 ZHANG, X. et al. Cucumber mosaic virus-encoded 2b suppressor inhibits Arabidopsis Argonaute1 cleavage activity to counter plant defense. **Genes & Development**, v. 20, n. 23, p. 3255–3268, 2006.

APÊNDICE A



Supplementary Figure SF1 Pineapple plant showing leaves (A) and lateral roots typical of healthy plants (B). Plant presenting reddish leaf, epinasty (C) and root death (D), MWP symptoms.

APÊNDICE B

Supplementary Table ST1. Differentially expressed genes in pineapple plants with MWP selected with cutoff of Log2FC > |0,7| and padj < 0.05.

(continua)

Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco008670	-4,12	AT4G11650	OSM34	osmotin 34 / thaumatin-like protein
Aco023684	-3,16	AT1G74950	TIFY10B	TIFY domain/jasmonate-zim-domain protein 1 / JAZ2
Aco004215	-2,97	AT3G04720	PR4	pathogenesis-related 4
Aco005912	-2,1	AT2G27140	RTM2	HSP20-like chaperones superfamily protein / RTM2
Aco008673	-2,84	AT4G11650	OSM34	osmotin 34
Aco005546	-2,54	AT5G24090	CHIA	chitinase A
Aco008200	-2,7	AT1G80840	WRKY40	WRKY DNA-binding protein 40
Aco020435	-1,69	AT4G27670	HSP21	heat shock protein 21
Aco016970	-2,27	AT3G07600	AT3G07600.1	NBS-LRR class disease resistance protein / Copper transport protein
Aco023187	2,26	AT5G27260	AT5G27260.1	Myb/SANT-like DNA-binding domain protein
Aco007169	2,03	AT2G44670	AT2G44670.1	senescence-associated family protein (DUF581)
Aco019805	-2,2	AT5G40010	AATP1	AAA-ATPase 1
Aco002888	1,96	AT3G01180	SS2	starch synthase 2
Aco000800	-2,32	-	-	hypothetical protein
Aco002069	-1,96	AT3G20570	ENODL9	early nodulin-like protein 9
Aco020932	-1,9	AT1G08280	GALT29A	Glycosyltransferase family 29 (sialyltransferase) family protein
Aco004204	-1,78	AT3G04620	DAN1	Target promoter of the male germline-specific transcription factor DUO1.
Aco002318	-1,87	AT2G41200	AT2G41200.1	transmembrane protein
Aco013232	1,37	AT1G79730	ELF7	hydroxyproline-rich glycoprotein family protein
Aco031642	-1,64	AT2G25520	AT2G25520.1	Drug/metabolite transporter superfamily protein
Aco005487	1,46	AT3G61680	PLIP1	PLASTID LIPASE1, PLIP1 encodes a plastid localized phospholipase A1 involved in seed oil biosynthesis.
Aco007907	-1,5	AT4G33490	AT4G33490.2	Eukaryotic aspartyl protease family protein
Aco001358	1,36	AT1G05010	ACO4	Encodes 1-aminocyclopropane-1-carboxylate oxidase
Aco004677	-2	AT5G40780	LHT1	lysine histidine transporter 1
Aco012494	-1,85	AT3G12750	ZIP1	zinc transporter 1 precursor
Aco014784	-1,91	AT2G02010	GAD4	glutamate decarboxylase 4
Aco017899	-1,71	AT2G41380	AT2G41380.1	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Aco007632	-2,01	AT5G42020	BIP2	Heat shock protein 70 (Hsp 70) family protein

(continuação)

Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco011530	-1,34	AT1G80460	NHO1	Actin-like ATPase superfamily protein
Aco021110	-1,65	AT3G57030	AT3G57030.1	Calcium-dependent phosphotriesterase superfamily protein
Aco016766	1,29	AT5G24470	PRR5	pseudo-response regulator 5
Aco028861	-1,26	-	-	hypothetical protein
Aco029197	1,21	AT5G62000	ARF2	auxin response factor 2
Aco000963	-1,91	AT1G24020	MLP423	MLP-like protein 423
Aco014337	-1,53	AT1G14360	UTR3	UDP-galactose transporter 3
Aco006242	-2,07	AT5G44130	FLA13	FASCICLIN-like arabinogalactan protein 13 precursor
Aco011170	-1,86	AT4G20780	CML42	calmodulin like 42
Aco017741	1,46	AT5G51820	PGM	Phosphoglucomutase
Aco013116	1,26	AT5G47040	LON2	lon protease 2
Aco023992	1,53	AT1G32900	GBSS1	UDP-Glycosyltransferase superfamily protein
Aco008027	-1,82	AT5G16970	ERA	alkenal reductase
Aco013456	-1,56	AT1G75620	AT1G75620.1	glyoxal oxidase-related protein
Aco005719	-2,01	AT5G64810	WRKY51	WRKY DNA-binding protein 51
Aco002889	1,33	AT5G03760	CSLA9	Nucleotide-diphospho-sugar transferases superfamily protein
Aco011802	-1,45	AT2G47730	GSTF8	glutathione S-transferase phi 8
Aco000308	-1,63	AT2G20562	TAX2	Encodes a putative signalling peptide with similarity to TAX1. No known function has been demonstrated yet.
Aco007375	-1,37	AT1G02640	BXL2	beta-xylosidase 2
Aco014868	-1,79	AT3G20570	ENODL9	early nodulin-like protein 9
Aco029271	-1,65	AT3G20570	ENODL9	early nodulin-like protein 9
Aco020133	-1,56	AT3G48360	BT2	BTB and TAZ domain protein 2
Aco009674	-1,69	AT1G04220	KCS2	3-ketoacyl-CoA synthase 2
Aco010159	-1,23	AT5G18460	AT5G18460.1	carboxyl-terminal peptidase (DUF239)
Aco002008	-1,38	-	-	hypothetical protein
Aco013060	-1,79	AT3G12500	PR3	basic chitinase
Aco013453	-1,23	AT1G75620	AT1G75620.1	glyoxal oxidase-related protein
Aco013460	-1,35	AT1G75620	AT1G75620.1	glyoxal oxidase-related protein
Aco031530	1,52	AT3G20050	TCP-1	T-complex protein 1 alpha subunit
Aco016861	-1,66	AT3G54040	AT3G54040.1	PAR1 protein
Aco002476	-1,47	AT4G15920	SWEET17	Encodes a vacuolar fructose transporter expressed in parenchyma and xylem that controls leaf fructose content. When its expression is reduced, fructose accumulates in leaves.
Aco009267	1,16	AT3G19990	AT3G19990.1	E3 ubiquitin-protein ligase
Aco025751	-1,18	AT3G48460	SFAR4	SEED FATTY ACID REDUCER 4,GDSL-motif.
Aco009484	-1,31	AT5G21950	AT5G21950.1	alpha/beta-Hydrolases superfamily protein

(continuação)

Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco005158	-1,45	AT2G27140	AT2G27140.1	HSP20-like chaperones superfamily protein
Aco010674	-1,18	AT5G45910	AT5G45910.1	GDSL-motif esterase/acyltransferase/lipase.
Aco012424	-1,12	AT5G15630	COBL4	COBRA-like extracellular glycosyl-phosphatidyl inositol-anchored protein family
Aco003824	1,52	AT5G67385	AT5G67385.1	Phototropic-responsive NPH3 family protein
Aco003985	1,27	AT2G26250	KCS10	3-ketoacyl-CoA synthase 10
Aco005743	-1,16	AT2G26070	RTE1	Protein of unknown function (DUF778)
Aco015618	1,25	AT4G31840	ENODL15	early nodulin-like protein 15
Aco004216	-1,74	AT5G43700	ATAUX2-11	AUX/IAA transcriptional regulator family protein
Aco001848	-1,75	AT4G15910	DI21	drought-induced 21
Aco008856	-1,08	AT1G75900	AT1G75900.1	GDSL-like Lipase/Acylhydrolase superfamily protein
Aco003609	-1,21	AT1G28570	AT1G28570.1	SGNH hydrolase-type esterase superfamily protein
Aco008411	-1,11	AT5G11720	AGLU1	Glycosyl hydrolases family 31 protein
Aco011308	1,06	AT2G13610	ABCG5	ABC-2 type transporter family protein
Aco024248	-1,73	AT5G42020	BIP2	Heat shock protein 70 (Hsp 70) family protein
Aco004502	-1,29	AT1G14420	AT59	Pectate lyase family protein
Aco021994	1,52	AT5G26770	AT5G26770.1	myosin heavy chain, cardiac protein
Aco027180	-1,1	AT5G26660	MYB86	myb domain protein 86
Aco006817	-1,08	AT2G25737	AT2G25737.1	Sulfite exporter TauE/SafE family protein
Aco000315	-1,49	AT1G67150	AT1G67150.3	Plant protein of unknown function (DUF247) transmembrane
Aco020108	1,45	AT2G46530	ARF11	auxin response factor 11
Aco000801	-1,67	AT4G22370	AT4G22370.1	transmembrane protein
Aco012629	-1,3	AT3G54320	WRI1	Integrase-type DNA-binding superfamily protein
Aco010671	-1,56	AT2G31090	TAX1	Encodes a signalling peptide influencing lateral organ separation.
Aco012715	0.97	AT1G26110	DCP5	decapping 5
Aco007661	-0,98	AT5G08370	AGAL2	alpha-galactosidase 2
Aco003823	1,13	AT5G67390	AT5G67390.1	glycosyltransferase-like protein
Aco014362	1,69	AT5G62550	AT5G62550.1	microtubule-associated futsch-like protein
Aco007089	-1,42	AT5G05390	LAC12	laccase 12
Aco013356	1,45	AT3G49680	BCAT3	branched-chain aminotransferase 3
Aco018061	1,23	AT5G60450	ARF4	auxin response factor 4
Aco002549	-1,04	AT5G55590	QRT1	Pectin lyase-like superfamily protein
Aco012439	-1,3	AT5G55590	QRT1	Pectin lyase-like superfamily protein
Aco009293	-1,05	AT5G42020	BIP	Heat shock protein 70 (Hsp 70) family protein

(continuação)

Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco016923	-1,14	AT2G25110	SDF2	stromal cell-derived factor 2-like protein precursor
Aco009967	-1,03	AT5G25880	NADP-ME3	NADP-malic enzyme 3
Aco025533	-1,05	AT5G33370	CUS2	CUTIN SYNTHASE2
Aco002200	1,04	AT5G22000	RHF2A	RING-H2 group F2A
Aco013319	-1,63	AT5G42650	AOS	allene oxide synthase
Aco003200	-1,59	AT1G71695	AT1G71695.1	Peroxidase superfamily protein
Aco017705	-1,41	AT1G44575	NPQ4	Chlorophyll A-B binding family protein
Aco019829	-1,24	AT3G51030	TRX1	thioredoxin H-type 1
Aco021295	-1,44	AT3G07990	SCPL27	serine carboxypeptidase-like 27
Aco016746	-1,23	AT5G04770	CAT6	cationic amino acid transporter 6
Aco014740	-1,07	AT5G64300	GCH	GTP cyclohydrolase II
Aco015104	-1,37	AT1G26310	AGL10	K-box region and MADS-box transcription factor family protein
Aco011495	0,99	AT1G53050	AT1G53050.1	Protein kinase superfamily protein
Aco012869	-1,27	AT4G17550	G3PP4	Encodes a member of the phosphate starvation-induced glycerol-3-phosphate permease gene family
Aco002376	1,57	AT3G20060	UBC19	ubiquitin-conjugating enzyme19
Aco009846	1,59	AT5G62550	AT5G62550.1	microtubule-associated futsch-like protein
Aco017094	1,11	AT2G44190	EDE1	Family of unknown function (DUF566)
Aco002082	1,02	AT1G60420	NRX1	NUCLEOREDOXIN 1
Aco010380	-1,44	AT4G35970	APX5	ascorbate peroxidase 5
Aco007785	-1,09	-	-	hypothetical protein
Aco023355	-1,31	AT5G18970	AT5G18970.1	AWPM-19-like family protein
Aco024459	-1,31	AT3G18260	RTNLB9	Reticulon family protein. RETICULON-LIKE B 9
Aco019813	-1,08	-	-	hypothetical protein / Cysteine-rich transmembrane CYSTM domain
Aco027598	-1,33	-	-	hypothetical protein
Aco001569	-1,37	AT3G55990	ESK1	Plant protein of unknown function (DUF828)
Aco011124	-1,3	AT5G05340	PRX52	PEROXIDASE 52
Aco017498	-1,53	AT4G14270	AT4G14270.1	Protein containing PAM2 motif which mediates interaction with the PABC domain of polyadenyl binding proteins.
Aco004077	-0,97	AT5G09430	AT5G09430.1	alpha/beta-Hydrolases superfamily protein
Aco012948	1,1	AT5G16750	TOZ	Transducin family protein / WD-40 repeat family protein
Aco006889	-1,05	AT5G04250	AT5G04250.2	Cysteine proteinases superfamily protein
Aco005696	-1,53	AT5G10830	AT5G10830.1	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Aco011175	-1,43	AT2G38640	AT2G38640.1	LURP-one-like protein (DUF567)
Aco021122	-1,27	AT1G20450	ERD10	Dehydrin family protein

(continuação)

Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco013924	-1,55	AT1G10360	GSTU18	glutathione S-transferase TAU 18
Aco009653	1,26	AT5G28640	AN3	SSXT family protein
Aco008852	1,37	AT5G65660	AT5G65660.1	hydroxyproline-rich glycoprotein family protein
Aco016422	-0,92	-	-	hypothetical protein
Aco017801	-0,92	AT1G27440	IRX10	Exostosin family protein
Aco019042	0,91	AT1G67310	AT1G67310.1	Calmodulin-binding transcription activator protein with CG-1 and Ankyrin domains
Aco009763	-1,07	AT5G55190	RAN3	RAN GTPase 3
Aco003551	-0,93	AT1G66240	ATX1	homolog of anti-oxidant 1
Aco015914	-1,04	AT3G22400	LOX5	PLAT/LH2 domain-containing lipoxygenase family protein
Aco000049	0,97	AT4G03090	AT4G03090.2	sequence-specific DNA binding;sequence-specific DNA binding transcription factors
Aco017440	-1,14	AT2G23970	AT2G23970.1	Class I glutamine amidotransferase-like superfamily protein
Aco018754	1,03	AT1G25540	PFT1	phytochrome and flowering time regulatory protein (PFT1)
Aco026790	1,06	AT1G21610	AT1G21610.3	wound-responsive family protein
Aco008585	-0,95	AT3G50760	GATL2	galacturonosyltransferase-like 2
Aco018301	-1,29	AT2G32300	UCC1	uclacyanin 1
Aco005229	-1,11	AT1G04240	SHY2	AUX/IAA transcriptional regulator family protein
Aco001800	-1,16	AT5G49350	AT5G49350.1	Glycine-rich protein family
Aco011154	-1,22	AT5G01930	MAN6	ENDO-BETA-MANNASE 6
Aco014229	1,51	AT5G02600	AT5G02600.2	Encodes a phloem mobile metal binding protein necessary for phloem function and root meristem maintenance.
Aco002747	-1,11	AT4G24380	AT4G24380.1	dihydrofolate reductase
Aco022141	-1,42	AT1G75900	AT1G75900.1	GDSL-like Lipase/Acylhydrolase superfamily protein
Aco015095	0,97	AT1G27340	LCR	LEAF CURLING RESPONSIVENESS. Encodes a putative F-box protein that is involved in the regulation of leaf morphology.
Aco012097	-1,07	AT4G28050	TET7	tetraspanin7
Aco013532	-0,89	AT1G67750	AT1G67750.1	Pectate lyase family protein
Aco022487	-1,39	AT1G35180	TRAM	TRAM, LAG1 and CLN8 (TLC) lipid-sensing domain containing protein
Aco010248	-1,49	AT2G36690	AT2G36690.1	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
Aco007985	0,98	AT2G30390	FC2	ferrochelatase 2
Aco008255	-1,46	AT4G25830	CASPL2C1	Uncharacterised protein family (UPF0497)
Aco022722	0,94	AT4G00930	CIP4.1	COP1-interacting protein 4.1
Aco001922	0,89	AT5G27120	AT5G27120.1	SAR DNA-binding protein, putative

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Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco021391	-1,47	-	-	hypothetical protein
Aco014440	1,15	AT4G21380	RK3	receptor kinase 3
Aco007930	-0,95	AT1G77020	AT1G77020.1	DNAJ heat shock N-terminal domain-containing protein
Aco007366	-1,05	AT1G09210	CRT1b	calreticulin 1b
Aco015579	1,01	AT4G11670	AT4G11670.1	DNA topoisomerase 4 subunit B (DUF810)
Aco026248	-1,35	AT1G55260	LTPG6	GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED LIPID PROTEIN TRANSFER 6
Aco021354	-1,4	AT5G06730	AT5G06730.1	Peroxidase superfamily protein
Aco024040	-1,34	AT5G46530	AT5G46530.1	AWPM-19-like family protein
Aco007718	-1,41	AT2G37170	PIP2	plasma membrane intrinsic protein 2
Aco005261	-1,18	AT3G51030	TRX1	thioredoxin H-type 1
Aco014318	1,39	AT1G80300	NTT1	nucleotide transporter 1
Aco012326	-1	-	-	hypothetical protein
Aco007909	1,08	AT5G63960	EMB2780	DNA binding;nucleotide binding;nucleic acid binding;DNA-directed DNA polymerases;DNA-directed DNA polymerases
Aco003460	-1,2	AT3G22400	LOX5	PLAT/LH2 domain-containing lipoxygenase family protein
Aco007701	1,09	AT2G22560	NET2D	NETWORKED 2D. Kinase interacting (KIP1-like) family protein
Aco010492	1	AT3G21690	AT3G21690.1	MATE efflux family protein
Aco008380	1,07	AT1G06570	PDS1	phytoene desaturation 1
Aco003574	-1,2	AT4G35350	XCP1	xylem cysteine peptidase 1
Aco009454	-1	AT4G03390	SRF3	STRUBBELIG-receptor family 3
Aco003241	0,89	AT1G47570	AT1G47570.1	RING/U-box superfamily protein
Aco002767	0,91	AT1G22460	AT1G22460.1	O-fucosyltransferase family protein
Aco008196	1,01	AT2G25180	RR12	response regulator 12
Aco001982	-1,1	AT5G04080	ATHCYSTM12	CYSTEINE-RICH TRANSMEMBRANE MODULE 12. cysteine-rich TM module stress tolerance protein.
Aco013004	-0,94	AT2G19690	PLA2-BETA	phospholipase A2-beta
Aco003439	1,04	AT3G25500	FH1	formin homology 1
Aco015519	-1,27	AT5G13900	AT5G13900.1	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein
Aco028338	1,29	AT5G47020	AT5G47020.1	MraZ
Aco012174	-1,2	AT5G20810	SAUR70	SAUR-like auxin-responsive protein family
Aco015326	-1,34	AT1G50590	AT1G50590.1	RmlC-like cupins superfamily protein
Aco009501	-0,85	AT5G20720	CPN20	chaperonin 20
Aco014647	-1,31	AT2G36830	GAMMA-TIP	gamma tonoplast intrinsic protein
Aco005860	-1,05	AT5G57000	AT5G57000.2	DEAD-box ATP-dependent RNA helicase

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Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco005513	1,1	AT5G53160	RCAR3	regulatory components of ABA receptor 3
Aco021903	1,1	AT1G14610	TWN2	valyl-tRNA synthetase / valine--tRNA ligase (VALRS)
Aco027352	1,41	AT4G17750	HSF1	heat shock factor 1
Aco021582	-1,14	AT5G33370	CUS2	GDSL-motif esterase/acyltransferase/lipase.
Aco015813	-0,87	AT1G65295	AT1G65295.1	ubiquitin carboxyl-terminal hydrolase
Aco003847	-0,87	AT2G03350	AT2G03350.1	Protein of unknown function, DUF538
Aco015990	1,03	-	-	hypothetical protein
Aco004896	-0,88	-	-	Glycosyl transferase ceramide glucosyltransferase
Aco004430	-1,4	AT3G16240	AQP1	delta tonoplast integral protein
Aco007383	-1	AT3G62600	ERDJ3B	DNAJ heat shock family protein
Aco023409	-1,08	AT1G12780	UGE1	UDP-D-glucose/UDP-D-galactose 4-epimerase 1
Aco023809	1,14	AT3G43540	AT3G43540.1	initiation factor 4F subunit (DUF1350)
Aco013770	0,95	AT1G67190	AT1G67190.2	F-box/RNI-like superfamily protein
Aco004578	-1,08	AT3G02800	PFA-DSP3	PLANT AND FUNGI ATYPICAL DUAL-SPECIfiCITY. PHOSPHATASE 3
Aco013707	-1,21	AT3G17020	AT3G17020.1	Adenine nucleotide alpha hydrolases-like superfamily protein
Aco030748	-1,38	AT5G06730	AT5G06730.1	Peroxidase superfamily protein
Aco004621	-0,94	AT4G26510	UKL4	uridine kinase-like 4
Aco016540	-1,37	AT1G12940	,NRT2.5	nitrate transporter2.5
Aco012827	-1,3	AT4G17360	AT4G17360.1	Formyl transferase
Aco004820	-1,03	AT1G27350	AT1G27350.1	Ribosome associated membrane protein RAMP4
Aco010861	0,92	AT1G60490	VPS34	vacuolar protein sorting 34
Aco013817	0,94	AT4G11720	HAP2	hapless 2
Aco014480	-1,23	AT4G03500	AT4G03500.1	Ankyrin repeat family protein
Aco030245	1,31	AT1G79730	ELF7	hydroxyproline-rich glycoprotein family protein
Aco019039	1,24	AT5G15800	AGL2	K-box region and MADS-box transcription factor family protein
Aco009519	-1,26	AT5G20870	AT5G20870.1	O-Glycosyl hydrolases family 17 protein
Aco014850	-1,32	-	-	Polyphenol oxidase I
Aco021381	-1,34	AT5G46060	AT5G46060.1	Protein of unknown function, DUF599
Aco000414	0,97	AT5G50340	AT5G50340.1	DNA repair protein RadA-like protein
Aco020843	-1,36	AT2G23910	AT2G23910.1	NAD(P)-binding Rossmann-fold superfamily protein
Aco010167	-1,1	AT2G20190	CLASP	CLIP-associated protein
Aco011109	-1,34	AT2G38470	WRKY33	WRKY DNA-binding protein 33
Aco012547	-1,31	AT5G05270	CHIL	CHALCONE ISOMERASE LIKE.
Aco016612	-1,03	-	-	hipothetical protein
Aco006593	-1,1	AT4G20050	QRT3	Pectin lyase-like superfamily protein

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Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco005598	-0,95	AT4G18950	BHP1	BLUE LIGHT SIGNALING1
Aco005369	-1,35	AT5G03260	LAC11	laccase 11
Aco019331	-0,84	AT4G28290	AT4G28290.1	hypothetical protein
Aco027950	-1,27	AT2G38470	WRKY33	WRKY DNA-binding protein 33
Aco005557	-0,88	AT4G28000	AT4G28000.1	P-loop containing nucleoside triphosphate hydrolases superfamily protein
Aco021905	-0,98	AT4G32390	AT4G32390.1	Nucleotide-sugar transporter family protein
Aco025000	1,02	AT4G27010	EMB2788	EMBRYO DEFECTIVE 2788
Aco001732	-0,92	AT2G27030	CAM5	calmodulin 5
Aco024025	0,95	AT2G05760	NAT1	NUCLEOBASE ASCORBATE TRANSPORTER 1
Aco005458	1,03	AT1G80300	NTT1	nucleotide transporter 1
Aco007676	-1,11	AT5G23350	AT5G23350.1	GRAM domain-containing protein / ABA-responsive protein-related
Aco014421	-1,06	AT1G50180	AT1G50180.1	NB-ARC domain-containing disease resistance protein
Aco013584	0,88	AT1G74880	NDH-O	NAD(P)H:plastoquinone dehydrogenase complex subunit O
Aco012992	-1	AT1G65840	PAO4	polyamine oxidase 4
Aco019032	0,96	AT5G51300	ATSF1	ARABIDOPSIS SF1 HOMOLOG
Aco022396	0,8	AT5G33280	CLCG	CHLORIDE CHANNEL G
Aco003751	-1,33	AT1G18760	AT1G18760.1	Zinc finger, C3HC4 type (RING finger) family protein
Aco001605	-0,81	AT2G38480	CASPL4B1	CASP-LIKE PROTEIN 4B1
Aco011461	0,84	AT1G33410	SAR1	SUPPRESSOR OF AUXIN RESISTANCE1
Aco009579	-1,18	AT2G22240	MIPS2	myo-inositol-1-phosphate synthase 2
Aco014159	0,8	AT1G06150	EMB1444	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
Aco009148	0,98	AT4G03400	DFL2	Auxin-responsive GH3 family protein
Aco002572	-1,09	AT4G01950	GPAT3	glycerol-3-phosphate acyltransferase 3
Aco009177	1,29	-	-	hypothetical protein
Aco005240	0,85	AT3G04670	WRKY39	WRKY DNA-binding protein 39
Aco005265	-1,06	AT1G75450	CKX5	cytokinin oxidase 5
Aco006051	-1,24	AT5G25610	RD22	BURP domain-containing protein
Aco007666	0,86	AT5G23390	AT5G23390.1	polygalacturonase inhibitor (DUF639)
Aco012834	1,1	-	-	hypothetical protein
Aco024235	-0,97	AT5G26330	AT5G26330.1	Cupredoxin superfamily protein
Aco015554	-0,89	AT1G12570	AT1G12570.1	Ortholog of maize IPE1 gene which is involved in pollen exine development.
Aco015070	0,83	AT5G13980	AT5G13980.1	Glycosyl hydrolase family 38 protein
Aco002014	0,8	AT1G11480	AT1G11480.1	eukaryotic translation initiation factor-related
Aco018946	-0,93	AT2G32990	GH9B8	glycosyl hydrolase 9B8

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Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco009241	-1,01	AT3G19900	AT3G19900.1	hypothetical protein
Aco002312	0,78	AT1G26540	AT1G26540.1	Agenet domain-containing protein
Aco009143	-1,08	AT1G07180	NDA1	alternative NAD(P)H dehydrogenase 1
Aco011167	-0,87	AT5G01990	PILS6	Auxin efflux carrier family protein
Aco002529	-1,17	AT1G56320	AT1G56320.1	hypothetical protein
Aco011477	-0,83	AT5G67230	IRX14-L	Nucleotide-diphospho-sugar transferases superfamily protein
Aco002247	-0,8	AT5G64510	TIN1	TUNICAMYCIN INDUCED 1
Aco002409	-1,13	-	-	hipothetical protein
Aco018619	1,01	AT2G05210	ATPOT1	Nucleic acid-binding, OB-fold-like protein
Aco020351	-0,91	-	-	hypothetical protein
Aco000477	0,88	AT5G01260	AT5G01260.2	Carbohydrate-binding-like fold
Aco008054	-0,95	AT2G43320	AT2G43320.2	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Aco019877	-1,14	AT2G41640	AT2G41640.1	Glycosyltransferase family 61 protein
Aco011335	-0,9	AT4G10040	CYTC-2	cytochrome c-2
Aco001824	-1,23	AT2G40330	PYL6	PYR1-like 6
Aco024639	-1,16	AT2G02850	ARPN	Plantacyanin
Aco016989	-0,99	AT1G32170	XTH30	xyloglucan endotransglucosylase/hydrolase 30
Aco009133	-1	AT1G11260	STP1	sugar transporter 1
Aco013327	-0,91	AT2G23760	BLH4	BEL1-like homeodomain 4
Aco014114	-1,09	AT2G38080	LMCO4	Laccase/Diphenol oxidase family protein
Aco023142	-0,91	AT5G59810	SBT5.4	Subtilase family protein
Aco008245	-0,79	-	-	hypothetical protein
Aco001653	-1,22	AT2G38600	AT2G38600.1	HAD superfamily, subfamily IIIB acid phosphatase
Aco003358	0,87	AT5G62000	ARF2	auxin response factor 2
Aco014258	1,16	-	-	uncharacterized protein
Aco013429	-1,24	AT5G55240	ATPXG2	ARABIDOPSIS THALIANA PEROXYGENASE 2
Aco010813	0,9	AT3G16260	TRZ4	tRNAse Z4
Aco014996	-0,97	AT3G43790	ZIFL2	zinc induced facilitator-like 2
Aco010948	-0,91	AT1G47278	AT1G47278.2	hypothetical protein
Aco013085	-0,78	AT4G35840	AT4G35840.1	RING/U-box superfamily protein
Aco001709	-0,9	AT3G56490	HIT3	HIS triad family protein 3
Aco018494	0,9	AT5G48310	RASD1	RESPONSIVENESS TO ABA SALT AND DROUGHT 1
Aco016348	-0,76	AT1G52910	AT1G52910.1	fiber (DUF1218)
Aco021409	0,93	AT4G14180	PRD1	putative recombination initiation defect 1
Aco016219	1,15	-	-	hypothetical protein
Aco000472	-1,03	AT5G01410	PDX1	Aldolase-type TIM barrel family protein
Aco011558	0,93	AT4G31020	AT4G31020.2	alpha/beta-Hydrolases superfamily protein

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Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco028950	0,89	AT5G27110	AT5G27110.1	Tetratricopeptide repeat (TPR)-like superfamily protein
Aco014475	-1,06	AT4G03500	AT4G03500.1	Ankyrin repeat family protein
Aco021005	-0,8	AT5G43060	RD21B	ESPONSIVE TO DEHYDRATION 21B. Peptidase, activity detected in extracts of root, leaf and cell culture.
Aco000930	-1,01	AT5G20090	AT5G20090.1	MPC1 negatively regulates ABA enhanced slow anion channel function during stomatal closure.
Aco005603	-0,97	AT1G78580	TPS1	trehalose-6-phosphate synthase
Aco002177	0,91	AT1G58030	CAT2	cationic amino acid transporter 2
Aco005230	-0,91	AT4G14550	IAA14	indole-3-acetic acid inducible 14
Aco006636	-0,8	AT5G55160	SUMO2	small ubiquitin-like modifier 2
Aco003312	-1,26	AT4G03520	ATHM2	Thioredoxin superfamily protein
Aco013111	-1,14	AT4G34950	MFS1	MAJOR FACILITATOR SUPERFAMILY 1
Aco013223	0,8	AT1G33060	NAC014	NAC 014
Aco009825	-0,81	AT5G64780	AT5G64780.1	Uncharacterised conserved protein UCP009193
Aco015789	0,79	AT2G17520	IRE1-2	Endoribonuclease/protein kinase IRE1-like
Aco009213	-0,92	AT2G17500	PILS5	PIN-LIKES 5. Auxin efflux carrier family protein
Aco001963	-0,78	AT2G41430	ERD15	dehydration-induced protein
Aco027864	0,75	AT1G58030	CAT2	cationic amino acid transporter 2
Aco012262	0,88	AT5G14520	PES	PESCADILLO
Aco006132	-1,23	AT5G07490	AT5G07490.1	transmembrane protein
Aco008653	-1,2	AT2G35795	AT2G35795.1	Chaperone DnaJ-domain superfamily protein
Aco001603	-1,1	AT5G05390	LAC12	laccase 12
Aco011255	1	AT1G44760	AT1G44760.1	Adenine nucleotide alpha hydrolases-like superfamily protein
Aco021163	-1,25	AT5G26340	STP13	Major facilitator superfamily protein
Aco015349	-1,04	AT2G33630	AT2G33630.1	NAD(P)-binding Rossmann-fold superfamily protein
Aco004086	-1	AT1G78170	AT1G78170.1	E3 ubiquitin-protein ligase
Aco016356	-1,09	AT5G08380	AGAL1	alpha-galactosidase 1
Aco021999	1,03	AT3G62200	AT3G62200.1	Putative endonuclease or glycosyl hydrolase
Aco000159	-0,88	AT3G12587	AT3G12587.1	Oligosaccaryltransferase
Aco024440	0,84	AT4G10380	NIP5;1	NOD26-like intrinsic protein 5;1
Aco002988	-0,89	AT5G37690	AT5G37690.1	SGNH hydrolase-type esterase superfamily protein
Aco003569	1,1	AT1G13030	COILIN	Encodes a plant coilin
Aco015215	1,04	AT1G10970	ATZIP4,ZIP4	zinc transporter 4 precursor
Aco025857	0,81	AT1G15690	AVP1	Inorganic H pyrophosphatase family protein
Aco003649	0,97	AT5G63540	RMI1	Domain of unknown function (DUF1767)

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Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco004976	0,83	AT2G14520	AT2G14520.1	CBS domain-containing protein with a domain of unknown function (DUF21)
Aco004246	-0,82	AT1G03070	AT1G03070.1	Bax inhibitor-1 family protein
Aco025845	-1,22	AT1G80060	AT1G80060.1	Ubiquitin-like superfamily protein
Aco002581	-1,23	-	-	hypothetical protein
Aco003287	0,82	AT1G77320	MEI1	transcription coactivators
Aco009774	0,97	AT1G80300	NTT1	nucleotide transporter 1
Aco011787	-0,85	AT2G17730	NIP2	NEP-interacting protein 2
Aco012868	-1,05	AT5G07475	AT5G07475.1	Cupredoxin superfamily protein
Aco013919	-0,95	AT1G10360	GSTU18	glutathione S-transferase TAU 18
Aco001218	-0,91	AT1G34670	MYB93	Encodes a member of the R2R3 transcription factor gene family that is a negative regulator of lateral root (LR) development.
Aco009433	-0,75	AT4G27745	AT4G27745.1	Yippee family putative zinc-binding protein
Aco014969	0,7	AT2G40960	AT2G40960.1	Single-stranded nucleic acid binding R3H protein
Aco018682	0,92	AT3G08860	PYD4	PYRIMIDINE 4
Aco020321	-1,02	AT5G56040	RGI4	RGF1 INSENSITIVE 4. STERILITY-REGULATING KINASE MEMBER 2. Leucine-rich receptor-like protein kinase family protein
Aco003844	-1,24	AT3G29240	AT3G29240.2	PPR containing protein (DUF179)
Aco031686	0,8	AT1G14400	UBC1	ubiquitin carrier protein 1
Aco008390	-1,15	AT3G05950	AT3G05950.1	RmlC-like cupins superfamily protein
Aco014187	-0,9	AT3G09390	MT2A	metallothionein 2ª
Aco017190	-0,74	AT3G05890	RCI2B	Low temperature and salt responsive protein family
Aco007848	-0,74	AT1G15120	AT1G15120.1	Ubiquinol-cytochrome C reductase hinge protein
Aco015073	0,74	AT5G62000	ARF2	auxin response factor 2
Aco016071	0,94	-	-	hypothetical protein
Aco022579	0,79	AT4G36720	HVA22K	HVA22-like protein K
Aco005951	-1,18	AT1G29930	CAB1	chlorophyll A/B binding protein 1
Aco007590	-1	AT5G09810	ACT7	actin 7
Aco016820	-1	AT5G02230	AT5G02230.1	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
Aco000444	-1,17	AT3G15990	SULTR3;4	sulfate transporter 3;4
Aco029630	1,22	AT2G07050	CAS1	cycloartenol synthase 1
Aco005366	0,75	AT4G36520	AUXILIN-LIKE4	Chaperone DnaJ-domain superfamily protein
Aco030613	1,19	AT5G57990	UBP23	ubiquitin-specific protease 23
Aco002052	0,97	AT5G06350	AT5G06350.1	ARM repeat superfamily protein
Aco000459	1,18	AT5G20060	AT5G20060.2	alpha/beta-Hydrolases superfamily protein

(conclusão)

Pineapple ID	Log2 FoldChange	TAIR10 ID	Symbol	Description (Phytozome, TAIR10 or NCBI database)
Aco010251	-0,91	AT4G00430	PIP1;4	plasma membrane intrinsic protein 1;4
Aco030438	-0,87	AT4G33640	AT4G33640.1	costars family protein
Aco019510	0,85	AT3G11964	AT3G11964.1	RIBOSOMAL RNA PROCESSING 5
Aco002817	-1,15	AT2G22170	PLAT2	PLAT DOMAIN PROTEIN 2. Lipase/lipooxygenase, PLAT/LH2 family protein
Aco015755	-0,86	AT4G37740	GRF2	growth-regulating factor 2
Aco011469	-1,19	AT1G44350	ILL6	IAA-leucine resistant (ILR)-like gene 6
Aco012406	-0,93	AT1G18650	PDCB3	plasmodesmata callose-binding protein 3
Aco013471	-0,85	AT1G65820	AT1G65820.2	microsomal glutathione s-transferase, putative
Aco015293	-0,86	AT1G68940	AT1G68940.1	Armadillo/beta-catenin-like repeat family protein
Aco016807	-0,8	AT2G39650	AT2G39650.1	cruciferin (DUF506)
Aco016390	-1,03	AT1G19600	AT1G19600.1	pfkB-like carbohydrate kinase family protein
Aco020840	-1,19	-	-	hypothetical protein
Aco017376	0,86	AT1G50910	AT1G50910.1	hypothetical protein
Aco014425	-0,93	AT1G11380	AT1G11380.1	PLAC8 family protein
Aco011651	-0,8	AT5G07440	GDH2	glutamate dehydrogenase 2
Aco006226	-1,09	AT4G18260	AT4G18260.1	Cytochrome b561/ferric reductase transmembrane protein family
Aco001133	-1,12	AT3G28857	PRE5	PACLOBUTRAZOL RESISTANCE 5. Encodes a atypical member of the bHLH (basic helix-loop-helix) family transcriptional factors.
Aco000314	0,76	AT1G30240	AT1G30240.2	proline-, glutamic acid/leucine-rich protein
Aco005293	-1	AT5G62680	GTR2	ATNPF2.11, GLUCOSINOLATE TRANSPORTER-2
Aco006766	-0,79	AT5G46340	AT5G46340.1	O-acetyltransferase family protein
Aco011060	-0,84	AT5G58600	PMR5	Plant protein of unknown function (DUF828)
Aco013954	-1,1	AT3G53420	PIP2A	plasma membrane intrinsic protein 2ª
Aco011001	-0,71	-	-	hypothetical protein
Aco008430	-0,88	AT4G21960	PRXR1	Peroxidase superfamily protein
Aco011237	-1,15	-	-	hypothetical protein
Aco011262	-0,93	AT1G10280	AT1G10280.1	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein
Aco007597	-0,73	AT5G23760	AT5G23760.1	Copper transport protein family
Aco014573	-0,81	AT3G07090	AT3G07090.1	PPPDE putative thiol peptidase family protein
Aco000252	1,06	AT5G57360	ZTL	Galactose oxidase/kelch repeat superfamily protein

APÊNDICE C

Supplementary Table ST2-A. DEGs cell function obtained by Mapman. (continua)

Cell Function	ID *Arabidospsis*	Description	Fold Change
aa ACTIVATION	at1g14610	TWN2 (TWIN 2); ATP binding / aminoacyl-tRNA ligase/ valine-tRNA ligase	1,1
CELL CYCLE	at1g77320	MEI1 (meiosis defective 1); transcription	0,82
PROTEIN DEGRADATION			
	at4g33490	aspartic-type endopeptidase	-1,5
Subtilases	at5g59810	SBT5.4; identical protein binding / serine-type endopeptidase	-0,91
cysteine protease	at4g35350	XCP1 (XYLEM CYSTEINE PEPTIDASE 1); endopeptidase	-1,2
	at5g04250	OTU-like cysteine protease family protein	-1,05
	at5g43060	cysteine proteinase, putative / thiol protease, putative	-0,8
serine protease	at5g47040	LON2 (LON PROTEASE 2); ATP binding	1,26
	at3g07990	SCPL27 (serine carboxypeptidase-like 27	-1,44
AAA type	at5g40010	AATP1 (AAA-ATPase 1); ATPase/ nucleoside-triphosphatase	-2,2
	at4g28000	ATP binding / ATPase/ nucleoside-triphosphatase	-0,88
Ubiquitin	at5g55160	SUMO2 (SMALL UBIQUITIN-LIKE MODIFIER 2); protein binding	-0,8
ubiquitin.E2	at3g20060	UBC19 (ubiquitin-conjugating enzyme19); ubiquitin-protein ligase	1,57
	at1g14400	UBC1 (UBIQUITIN CARRIER PROTEIN 1); ubiquitin-protein ligase	0,8
ubiquitin.E3.RING	at1g18760	zinc finger (C3HC4-type RING finger) family protein	-1,33
	at1g68940	armadillo/beta-catenin repeat protein-related	-0,86
	at4g35840	zinc finger (C3HC4-type RING finger) family protein	-0,78
	at2g17730	zinc finger (C3HC4-type RING finger) family protein	-0,85
	at1g47570	zinc finger (C3HC4-type RING finger) family protein	0,89
	at5g22000	RHF2A (RING-H2 GROUP F2A); protein binding / zinc ion binding	1,04
ubiquitin.E3.SCF.FBOX	at1g27340	F-box family protein	0,97
	at5g57360	ZTL (ZEITLUPE); protein binding / ubiquitin-protein	1,06
	at1g67190	F-box family protein	0,95
ubiquitin.E3.BTB/POZ	at3g48360	BT2 (BTB AND TAZ DOMAIN PROTEIN 2); transcription factor	-1,56
ubiquitin.protease	at5g57990	UBP23 (UBIQUITIN-SPECIFIC PROTEASE 23)	1,19

(continuação)

Cell Function	ID *Arabidospsis*	Description	Fold Change
DEVELOPMENT			
Unspecified	at1g75900	family II extracellular lipase 3 (EXL3)	-1,42
	at2g41380	embryo-abundant protein-related	-1,71
	at2g44670	senescence-associated protein-related	2,03
	at5g10830	embryo-abundant protein-related	-1,53
	at5g55240	caleosin-related family protein / embryo-specific protein, putative	-1,24
	at4g15920	nodulin MtN3 family protein	-1,47
	at3g54320	WRI1 (WRINKLED 1); DNA binding / transcription factor	-1,3
	at5g16750	TOZ (TORMOZEMBRYO DEFECTIVE); nucleotide binding	1,1
	at5g15800	AGL2 | SEP1 (SEPALLATA1); DNA binding / transcription factor	1,24
	at4g00930	CIP4.1	0,94
	at1g33060	ANAC014 | no apical meristem (NAM) family protein	0,8
	at5g14520	pescadillo-related	0,88
	at4g28050	TET7 (TETRASPANIN7)	-1,07
	at4g34950	nodulin family protein	-1,14
CELL DIVISION	at2g20190	CLASP (CLIP-ASSOCIATED PROTEIN); binding	-1,1
ENZYMES FAMILIES			
misc.UDP glucosyl and glucoronyl transferases	at3g50760	GATL2 (Galacturonosyltransferase-like 2); polygalacturonate 4-alpha-galacturonosyltransferase/ transferase, transferring glycosyl groups / transferase, transferring hexosyl groups	-0,95
	at5g67230	glycosyl transferase family 43 protein	-0,83
	at1g27440	IRX10, ATGUT1 | GUT2; catalytic/ glucuronoxylan glucuronosyltransferase	-0,92
misc.gluco-, galacto- and mannosidases	at5g20870	glycosyl hydrolase family 17 protein	-1,26
... and alpha-galactosidase	at5g11720	alpha-glucosidase 1 (AGLU1)	-1,11
... and alpha-mannosidase	at5g13980	glycosyl hydrolase family 38 protein	0,83
misc.beta 1,3 glucan hydrolases	at1g18650	PDCB3 (PLASMODESMATA CALLOSE-BINDING PROTEIN 3)	-0,93
misc.oxidases - copper, flavone etc.	at5g16970	AT-AER (alkenal reductase); 2-alkenal	-1,82
	at2g36690	oxidoreductase, 2OG-Fe(II) oxygenase family protein	-1,49
	at2g32300	UCC1 (UCLACYANIN 1); copper ion binding / electron carrier	-1,29

(continuação)

Cell Function	ID *Arabidospsis*	Description	Fold Change
ENZYMES FAMILIES			
misc.oxidases - copper, flavone etc.	at1g65840	ATPAO4 (ARABIDOPSIS THALIANA POLYAMINE OXIDASE 4)	-1
misc.glutathione S transferases	at1g65820	microsomal glutathione s-transferase, putative	-0,85
	at2g47730	ATGSTF5, GST6, GSTF8 | ATGSTF8 (ARABIDOPSIS THALIANA GLUTATHIONE S-TRANSFERASE PHI 8); glutathione transferase	-1,45
	at1g10360	ATGSTU18, GST29 | ATGSTU18 (GLUTATHIONE S-TRANSFERASE TAU 18); glutathione transferase	-1,55
misc.peroxidases	at5g05340	peroxidase, putative	-1,3
	at1g71695	peroxidase 12 (PER12) (P12) (PRXR6)	-1,59
	at4g21960	PRXR1; electron carrier/ heme binding / peroxidase	-0,88
	at5g06730	peroxidase, putative	-1,4
misc.acid and other phosphatases	at2g38600	acid phosphatase class B family protein	-1,22
misc.plastocyanin-like	at5g07475	plastocyanin-like domain-containing protein	-1,05
	at3g20570	plastocyanin-like domain-containing protein	-1,96
	at2g02850	ARPN (PLANTACYANIN); copper ion binding / electron carrier	-1,16
	at4g31840	plastocyanin-like domain-containing protein	1,25
	at5g26330	plastocyanin-like domain-containing protein / mavicyanin, putative	-0,97
misc.protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	at1g55260	lipid binding	-1,35
	at5g13900	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	-1,27
misc.GDSL-motif lipase	at5g37690	GDSL-motif lipase/hydrolase family protein	-0,89
	at1g28570	GDSL-motif lipase, putative	-1,21
	at3g48460	GDSL-motif lipase/hydrolase family protein	-1,18
	at5g33370	GDSL-motif lipase/hydrolase family protein	-1,14
	at5g45910	GDSL-motif lipase/hydrolase family protein	-1,18
HORMONES			
abscisic acid.induced-regulated-responsive-activated	at4g36720	HVA22K (HVA22-LIKE PROTEIN K)	0,79
	at5g23350	GRAM domain-containing protein / ABA-responsive protein-related	-1,11
auxin.synthesis-degradation	at1g44350	ILL6; IAA-amino acid conjugate hydrolase/ metallopeptidase	-1,19

(continuação)

Cell Function	ID *Arabidospsis*	Description	Fold Change
HORMONES			
auxin.induced-regulated-responsive-activated	at1g22460	unknown protein	0,91
	at5g20810	auxin-responsive protein, putative / small auxin up RNA (SAUR_B)	-1,2
	at4g03400	GH3-10 | DFL2 (DWARF IN LIGHT 2)	0,98
metabolism.brassinosteroid.synthesis-degradation.sterols.other	at2g07050	CAS1 (cycloartenol synthase 1)	1,22
metabolism.cytokinin.synthesis-degradation	at1g75450	CKX6 | CKX5 (CYTOKININ OXIDASE 5); cytokinin dehydrogenase	-1,06
cytokinin.signal transduction	at2g25180	ARR12 (ARABIDOPSIS RESPONSE REGULATOR 12); transcription factor/ two-component response regulator	1,01
ethylene.synthesis-degradation.1-aminocyclopropane-1-carboxylate oxidase	at1g05010	ACO4, EAT1 | EFE (ETHYLENE-FORMING ENZYME); 1-aminocyclopropane-1-carboxylate oxidase	1,36
metabolism.jasmonate.synthesis-degradation.lipoxygenase	at3g22400	LOX5; electron carrier/ iron ion binding / lipoxygenase/ metal ion binding / oxidoreductase, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen	-1,2
metabolism.jasmonate.synthesis-degradation.allene oxidase synthase	at5g42650	AOS, CYP74A, DDE2 | AOS (ALLENE OXIDE SYNTHASE); allene oxide synthase/ hydro-lyase/ oxygen binding	-1,63
METAL HANDLING			
	at3g07600	heavy-metal-associated domain-containing protein	-2,27
metal handling.binding, chelation and storage	at3g09390	MT2A (METALLOTHIONEIN 2A); copper ion binding	-0,9
	at1g66240	ATX1 (ARABIDOPSIS HOMOLOG OF ANTI-OXIDANT 1); metal ion binding	-0,93
PROTEIN MODIFICATION			
protein.postranslational modification	at2g17520	IRE1A, ATIRE1-2, IRE1-2 | IRE1A; endoribonuclease/ kinase	0,79
	at3g56490	zinc-binding protein, putative / protein kinase C inhibitor, putative	-0,9
	at1g53050	protein kinase family protein	0,99
protein.postranslational modification.kinase	at2g22560	kinase interacting family protein	1,09
CELL ORGANIZATION			
	at5g09810	ACT7 (ACTIN 7); structural constituent of cytoskeleton	-1
	at4g03500	ankyrin repeat family protein	-1,23
	at4g18950	ankyrin protein kinase, putative	-0,95

(continuação)

Cell Function	ID *Arabidospsis*	Description	Fold Change
RNA PROCESSING			
splicing	at5g51300	splicing factor-related	0,96
REDOX			
thioredoxin	at4g03520	ATHM2; enzyme activator	-1,26
	at3g51030	ATTRX H1 | ATTRX1; oxidoreductase, acting on sulfur group of donors, disulfide as acceptor	-1,24
ascorbate and glutathione	at4g18260	cytochrome B561-related	-1,09
ascorbate and glutathione.ascorbate	at4g35970	APX5 (ASCORBATE PEROXIDASE 5); L-ascorbate peroxidase/ heme binding / peroxidase	-1,44
REGULATION OF TRANSCRIPTION			
RNA.regulation of transcription	at1g79730	ELF7 (EARLY FLOWERING 7)	1,37
ARF, Auxin Response Factor family	at5g60450	ARF4 (AUXIN RESPONSE FACTOR 4); transcription factor	1,23
	at5g62000	ARF1-BP, HSS | ARF2 (AUXIN RESPONSE FACTOR 2); protein binding / transcription factor	1,21
	at2g46530	ARF11 (AUXIN RESPONSE FACTOR 11); transcription factor	1,45
ARR	at2g25180	ARR12 (ARABIDOPSIS RESPONSE REGULATOR 12); transcription factor/ two-component response regulator	1,01
bHLH,Basic Helix-Loop-Helix family	at1g06150	transcription factor	0,8
HB,Homeobox transcription factor family	at2g23760	SAW2 | BLH4 (BEL1-LIKE HOMEODOMAIN 4); DNA binding / transcription factor	-0,91
HSF,Heat-shock transcription factor family	at4g17750	HSF1 (HEAT SHOCK FACTOR 1); DNA binding / transcription factor	1,41
MADS box transcription factor family	at5g15800	AGL2 | SEP1 (SEPALLATA1); DNA binding / transcription factor	1,24
	at1g26310	CAL, CAL1, agl10 | CAL (CAULIFLOWER); DNA binding / transcription factor	-1,37
MYB domain transcription factor family	at1g34670	AtMYB93 (myb domain protein 93); DNA binding / transcription factor	-0,91
	at5g26660	ATMYB4 | ATMYB86 (MYB DOMAIN PROTEIN 86); specific transcriptional repressor/ transcription repressor	-1,1
WRKY domain transcription factor family	at3g04670	WRKY39; calmodulin binding / transcription factor	0,85
	at1g80840	WRKY40; transcription factor	-2,7
	at5g64810	WRKY51; transcription factor	-2,01

(continuação)

Cell Function	ID *Arabidospsis*	Description	Fold Change
REGULATION OF TRANSCRIPTION			
WRKY domain transcription factor family	at2g38470	WRKY33; transcription factor	-1,34
Aux/IAA family	at5g43700	IAA4 | ATAUX2-11 (AUXIN INDUCIBLE 2-11); DNA binding / transcription factor	-1,74
	at1g04240	IAA3 | SHY2 (SHORT HYPOCOTYL 2); transcription factor	-1,11
	at4g14550	SLR | IAA14 (INDOLE-3-ACETIC ACID INDUCIBLE 14); protein binding / transcription factor/ transcription repressor	-0,91
General Transcription	at4g37740	AtGRF2 (GROWTHREGULATING FACTOR 2); transcription activator	-0,86
Psudo ARR transcription factor family	at5g24470	PRR5 (ARABIDOPSIS PSEUDO-RESPONSE REGULATOR 5); transcription regulator/ two-component response regulator	1,29
putative transcription regulator	at5g27120	SAR DNA-binding protein, putative	0,89
REGULATION/SIGNALLING			
in sugar and nutrient physiology	at3g54040	photoassimilate-responsive protein-related	-1,66
receptor kinases.leucine rich repeat V	at4g03390	SRF3 (STRUBBELIG-RECEPTOR FAMILY 3); ATP binding / kinase/ protein serine/threonine kinase	-1
receptor kinases.leucine rich repeat XI	at5g56040	leucine-rich repeat protein kinase, putative	-1,02
receptor kinases.S-locus glycoprotein like	at4g21380	ARK3 (A. THALIANA RECEPTOR KINASE 3); kinase/ transmembrane receptor protein serine/threonine kinase	1,15
calcium	at2g27030	CAM5 (CALMODULIN 5); calcium ion binding	-0,92
	at1g09210	calreticulin 2 (CRT2)	-1,05
	at4g20780	calcium-binding protein, putative	-1,86
G-proteins	at5g55190	RAN3 (RAN GTPASE 3); GTP binding / GTPase/ protein binding	-1,07
lipids	at1g60490	VPS34; 1-phosphatidylinositol-3-kinase/ binding / inositol or phosphatidylinositol kinase/ phosphotransferase, alcohol group as acceptor	0,92
light	at1g25540	PFT1 (PHYTOCHROME AND FLOWERING TIME 1); transcription coactivator	1,03
	at5g67385	protein binding / signal transducer	1,52
	at1g67310	calmodulin binding / transcription regulator	0,91
DNA REPAIR			
	at1g77320	MEI1 (meiosis defective 1); transcription coactivator	0,82
	at5g50340	ATP binding / damaged DNA binding / nucleoside-triphosphatase	0,97

(continuação)

Cell Function	ID *Arabidospsis*	Description	Fold Change
PROTEIN SYNTHESIS			
initiation	at1g11480	eukaryotic translation initiation factor-related	0,8
DNA SYNTHESIS			
chromatin structure	at2g05210	ATPOT1 | AtPOT1a (Protection of Telomeres 1a); telomeric DNA binding	1,01
	at5g63960	EMB2780 (EMBRYO DEFECTIVE 2780); DNA binding / DNA-directed DNA polymerase/ nucleic acid binding / nucleotide binding	1,08
STRESS ABIOTIC			
	at4g11650	ATOSM34 (osmotin 34)	-4,12
heat	at5g42020	BIP, | BIP2; ATP binding	-2,01
	at1g77020	DNAJ heat shock N-terminal domain-containing protein	-0,95
	at2g27140	heat shock family protein	-2,1
	at2g35795	DNAJ heat shock N-terminal domain-containing protein	-1,2
	at4g27670	HSP21 (HEAT SHOCK PROTEIN 21)	-1,69
	at3g62600	ATERDJ3B; heat shock protein binding / unfolded protein binding	-1
cold	at3g17020	universal stress protein (USP) family protein	-1,21
drought/salt	at2g41430	LSR1, CID1 | ERD15 (EARLY RESPONSIVE TO DEHYDRATION 15); protein binding	-0,78
	at5g25610	RD22; nutrient reservoir	-1,24
	at3g05890	RCI2B (RARE-COLD-INDUCIBLE 2B)	-0,74
	at4g15910	DI21 | ATDI21 (ARABIDOPSIS THALIANA DROUGHT-INDUCED 21)	-1,75
touch/wounding	at1g21610	wound-responsive family protein	1,06
unspecified	at2g40330	Bet v I allergen family protein | chr2:16844864-16845934 REVERSE	-1,23
	at1g24020	MLP423 (MLP-LIKE PROTEIN 423)	-1,91
	at1g44760	universal stress protein (USP) family protein	1
	at1g20450	LTI29, LTI45, ERD10 (EARLY RESPONSIVE TO DEHYDRATION 10); actin binding	-1,27
	at3g05950	germin-like protein, putative	-1,15
STRESS BIOTIC	at3g12500	PR-3, CHI-B, ATHCHIB (ARABIDOPSIS THALIANA BASIC CHITINASE)	-1,79
	at3g04720	HEL, PR-4 (PATHOGENESIS-RELATED 4); chitin binding	-2,97

(continuação)

Cell Function	ID *Arabidospsis*	Description	Fold Change
STRESS BIOTIC			
PR-proteins	at1g50180	disease resistance protein (CC-NBS-LRR class), putative	-1,06
	at5g24090	acidic endochitinase (CHIB1)	-2,54
TRANSPORT			
Sugars	at1g11260	STP1 (SUGAR TRANSPORTER 1); carbohydrate transmembrane transporter/ sugar:hydrogen symporter	-1
	at5g26340	STP13 | MSS1; carbohydrate transmembrane transporter/ hexose:hydrogen symporter/ high-affinity hydrogen:glucose symporter/ sugar:hydrogen symporter	-1,25
amino acids	at1g58030	CAT2 (CATIONIC AMINO ACID TRANSPORTER 2); amino acid transmembrane transporter	0,91
	at5g40780	LHT1; amino acid transmembrane transporter	-2
	at5g04770	CAT6 (CATIONIC AMINO ACID TRANSPORTER 6); amino acid transmembrane transporter/ basic amino acid transmembrane transporter/ cationic amino acid transmembrane transporter	-1,23
Nitrate	at1g12940	ATNRT2.5 (nitrate transporter2.5); nitrate transmembrane transporter	-1,37
Sulphate	at3g15990	SULTR3;4 (SULFATE TRANSPORTER 3;4); sulfate transmembrane transporter	-1,17
metabolite transporters at the envelope membrane	at2g25520	phosphate translocator-related	-1,64
	at4g32390	phosphate translocator-related	-0,98
metabolite transporters at the mitochondrial membrane	at4g17550	transporter-related	-1,27
NDP-sugars at the ER	at1g14360	UTR3 (UDP-GALACTOSE TRANSPORTER 3); pyrimidine nucleotide sugar transmembrane transporter	-1,53
Metal	at3g12750	ZIP1 (ZINC TRANSPORTER 1 PRECURSOR); zinc ion transmembrane transporter	-1,85
	at1g10970	ZIP4 (ZINC TRANSPORTER 4 PRECURSOR); cation transmembrane transporter/ copper ion transmembrane transporter	1,04
peptides and oligopeptides	at5g62680	proton-dependent oligopeptide transport (POT) family protein	-1
ABC transporters and multidrug resistance systems	at2g13610	ABC transporter family protein	1,06
	at3g43790	ZIFL2 (ZINC INDUCED FACILITATOR-like 2); carbohydrate transmembrane transporter/ sugar:hydrogen symporter	-0,97

(continuação)

Cell Function	ID *Arabidospsis*	Description	Fold Change
TRANSPORT			
unspecified anions	at5g33280	chloride channel-like (CLC) protein, putative	0,8
Major Intrinsic Proteins.PIP	at3g53420	PIP2A (PLASMA MEMBRANE INTRINSIC PROTEIN 2A); water channel	-1,1
	at2g37170	PIP2B (PLASMA MEMBRANE INTRINSIC PROTEIN 2); water channel	-1,41
	at4g00430	PIP1;4 (PLASMA MEMBRANE INTRINSIC PROTEIN 1;4); water channel	-0,91
Major Intrinsic Proteins.TIP	at3g16240	DELTA-TIP; ammonia transporter/ methylammonium transmembrane transporter/ water channel	-1,4
	at2g36830	GAMMA-TIP (GAMMA TONOPLAST INTRINSIC PROTEIN); water channel	-1,31
Major Intrinsic Proteins.NIP	at4g10380	NIP5;1, NLM6, NLM8 ; arsenite transmembrane transporter/ boron transporter/ water channel	0,84
H+ transporting pyrophosphatase	at1g15690	ATAVP3, AVP-3 | AVP1; ATPase/ hydrogen-translocating pyrophosphatase	0,81
transport.misc	at3g21690	MATE efflux family protein	1
	at5g46530	AWPM-19-like membrane family	-1,34
	at5g01990	auxin efflux carrier family protein	-0,87
	at1g80300	| NTT1 (NUCLEOTIDE TRANSPORTER 1); ATP:ADP antiporter	1,39
	at5g18970	AWPM-19-like membrane family protein	-1,31
	at2g17500	auxin efflux carrier family protein	-0,92
	at2g05760	xanthine/uracil permease family protein	0,95
UNCLASSIFIED NO ONTOLOGY			
	at3g16260	TRZ4 (TRNASE Z 4); 3'-tRNA processing endoribonuclease/ catalytic	0,9
	at5g23760	heavy-metal-associated domain-containing protein	-0,73
	at1g30240	unknown protein	0,76
	at2g25110	SDF2 (STROMAL CELL-DERIVED FACTOR 2-LIKE PROTEIN PRECURSOR)	-1,14
	at5g46340	O-acetyltransferase-related	-0,79
	at2g20562	unknown protein	-1,63
	at1g47278	unknown protein	-0,91
	at4g36520	heat shock protein binding	0,75
	at4g25830	integral membrane family protein	-1,46
	at3g29240	unknown protein	-1,24

(continuação)

Cell Function	ID *Arabidospsis*	Description	Fold Change
UNCLASSIFIED NO ONTOLOGY	at5g09430	hydrolase	-0,97
	at1g12570	glucose-methanol-choline (GMC) oxidoreductase family protein	-0,89
	at2g23970	defense-related protein, putative	-1,14
	at2g14520	CBS domain-containing protein	0,83
	at5g02600	heavy-metal-associated domain-containing protein	1,51
	at5g21950	hydrolase, alpha/beta fold family protein	-1,31
	at1g03070	glutamate binding	-0,82
	at1g13030	sphere organelles protein-related	1,1
	at5g02230	haloacid dehalogenase-like hydrolase family protein	-1
	at3g18260	reticulon family protein (RTNLB9)	-1,31
	at2g38480	integral membrane protein, putative	-0,81
	at1g50590	pirin, putative	-1,34
	at2g22170	lipid-associated family protein	-1,15
agenet domain-containing protein	at1g26540	agenet domain-containing protein	0,78
pentatricopeptide (PPR) repeat-containing protein	at5g27110	pentatricopeptide (PPR) repeat-containing protein	0,89
S RNA-binding domain-containing protein	at3g11964	RNA binding	0,85
formin homology 2 domain-containing protein	at3g25500	FH1, AHF1, ATFH1 | AFH1 (FORMIN HOMOLOGY 1); actin binding / actin filament binding / protein binding	1,04
DC1 domain containing protein	at1g60420	DC1 domain-containing protein	1,02
glycine rich proteins	at5g49350	unknown protein	-1,16
	at5g47020	glycine-rich protein	1,29
	at5g28640	GIF, GIF1 | AN3 (ANGUSTIFOLIA 3); protein binding / transcription coactivator	1,26
hydroxyproline rich proteins	at5g65660	hydroxyproline-rich glycoprotein family protein	1,37
UNKNOWN	at1g10280	unknown protein	-0,93
	at1g11380	unknown protein	-0,93
	at1g26110	unknown protein	0,97
	at1g27350	unknown protein	-1,03
	at1g33410	NUP160, ATNUP160 | SAR1 (suppressor of auxin resistance1)	0,84

(continuação)

Cell Function	ID *Arabidospsis*	Description	Fold Change
UNKNOWN	at1g35180	unknown protein	-1,39
	at1g50910	unknown protein	0,86
	at1g52910	unknown protein	-0,76
	at1g56320	unknown protein	-1,17
	at1g65295	unknown protein	-0,87
	at1g67150	unknown protein	-1,49
	at1g74950	JAZ2, TIFY10B	-3,16
	at1g78170	unknown protein	-1
	at1g80060	unknown protein	-1,22
	at2g03350	unknown protein	-0,87
	at2g25737	unknown protein	-1,08
	at2g26070	RTE1 (REVERSION-TO-ETHYLENE SENSITIVITY1)	-1,16
	at2g31090	unknown protein	-1,56
	at2g38640	unknown protein	-1,43
	at2g39650	unknown protein	-0,8
	at2g41200	unknown protein	-1,87
	at2g41640	transferase, transferring glycosyl groups	-1,14
	at2g43320	unknown protein	-0,95
	at2g44190	EDE1 (ENDOSPERM DEFECTIVE 1); microtubule binding	1,11
	at3g02800	phosphatase/ phosphoprotein phosphatase/ protein tyrosine phosphatase	-1,08
	at3g04620	nucleic acid binding	-1,78
	at3g07090	unknown protein	-0,81
	at3g12587	unknown protein	-0,88
	at3g19900	unknown protein	-1,01
	at3g19990	unknown protein	1,16
	at3g28857	transcription regulator	-1,12
	at3g43540	unknown protein	1,14
	at3g55990	ESK1 (ESKIMO 1)	-1,37

(continuação)

Cell Function	ID *Arabidospsis*	Description	Fold Change
UNKNOWN	at3g62200	EDA32 (embryo sac development arrest 32)	1,03
	at4g03090	sequence-specific DNA binding / transcription factor	0,97
	at4g11670	unknown protein	1,01
	at4g11720	GCS1 | HAP2 (HAPLESS 2)	0,94
	at4g14180	AtPRD1 (Arabidopsis thaliana Putative Recombination initiation Defect 1); protein binding / protein homodimerization	0,93
	at4g14270	Protein containing PAM2 motif which mediates interaction with the PABC domain of polyadenyl binding proteins	-1,53
	at4g20050	QRT3 (QUARTET 3); polygalacturonase	-1,1
	at4g22370	unknown protein	-1,67
	at4g24380	unknown protein	-1,11
	at4g27010	unknown protein	1,02
	at4g27745	yippee family protein	-0,75
	at4g28290	unknown protein	-0,84
	at4g31020	unknown protein	0,93
	at4g33640	unknown protein	-0,87
	at5g04080	unknown protein	-1,1
	at5g06350	binding	0,97
	at5g07490	unknown protein	-1,23
	at5g18460	unknown protein	-1,23
	at5g20090	unknown protein	-1,01
	at5g23390	unknown protein	0,86
	at5g26770	unknown protein	1,52
	at5g27260	unknown protein	2,26
	at5g46060	unknown protein	-1,34
	at5g48310	unknown protein	0,9
	at5g53160	unknown protein	1,1
	at5g57000	unknown protein	-1,05
	at5g58600	PMR5 (POWDERY MILDEW RESISTANT 5)	-0,84

(conclusão)

Cell Function	ID *Arabidospsis*	Description	Fold Change
UNKNOWN	at5g62550	unknown protein	1,69
	at5g63540	unknown protein	0,97
	at5g64510	unknown protein	-0,8
	at5g64780	unknown protein	-0,81
	at5g67390	unknown protein	1,13

APÊNDICE D

Supplementary Table ST 2-B. DEGs mapped in Biotic stress categorie. (continua)

Categorie	ID *Arabidopsis*	Description	Fold Change
ABA			
abscisic acid.induced-regulated-responsive-activated	at4g36720	HVA22K (HVA22-LIKE PROTEIN K)	0,79
	at5g23350	GRAM domain-containing protein / ABA-responsive protein-related	-1,11
AUXIN			
auxin.synthesis-degradation	at1g44350	ILL6; IAA-amino acid conjugate hydrolase/ metallopeptidase	-1,19
auxin.induced-regulated-responsive-activated	at1g22460	unknown protein	0,91
	at5g20810	auxin-responsive protein, putative / small auxin up RNA (SAUR_B)	-1,2
	at4g03400	GH3-10 | DFL2 (DWARF IN LIGHT 2)	0,98
BRASSINOSTEROID			
brassinosteroid.synthesis-degradation.sterols.other	at2g07050	CAS1 (cycloartenol synthase 1)	1,22
ETHYLENE			
ethylene.synthesis-degradation.1-aminocyclopropane-1-carboxylate oxidase	at1g05010	ACO4, EAT1 | EFE (ETHYLENE-FORMING ENZYME); 1-aminocyclopropane-1-carboxylate oxidase	1,36
GLUTATHIONE			
misc.glutathione S transferases	at1g65820	microsomal glutathione s-transferase, putative	-0,85
	at2g47730	ATGSTF5, GST6, GSTF8 | ATGSTF8 (ARABIDOPSIS THALIANA GLUTATHIONE S-TRANSFERASE PHI 8); glutathione binding / glutathione transferase	-1,45
	at1g10360	GST29 | ATGSTU18 (GLUTATHIONE S-TRANSFERASE TAU 18); glutathione transferase	-1,55
HSPS			
stress.abiotic.heat	at5g42020	BIP, BIP2; ATP binding	-2,01
	at1g77020	DNAJ heat shock N-terminal domain-containing protein	-0,95
	at2g27140	heat shock family protein	-2,1
	at2g35795	DNAJ heat shock N-terminal domain-containing protein	-1,2
	at4g27670	HSP21 (HEAT SHOCK PROTEIN 21)	-1,69

(continuação)

Categorie	ID *Arabidopsis*	Description	Fold Change
HSPS			
stress.abiotic.heat	at3g62600	ATERDJ3B; heat shock protein binding / unfolded protein binding	-1
JASMONATE			
jasmonate.synthesis-degradatione	at3g22400	LOX5; electron carrier/ iron ion binding / lipoxygenase/ metal ion binding / oxidoreductase, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen	-1,2
	at5g42650	CYP74A, DDE2 | AOS (ALLENE OXIDE SYNTHASE); allene oxide synthase/ hydro-lyase/ oxygen binding	-1,63
SECUNDARY METABOLISM			
Isoprenoids	at2g26250	FDH, KCS10 (3-KETOACYL-COA SYNTHASE 10); acyltransferase/ catalytic/ transferase, transferring acyl groups other than amino-acyl groups	1,27
isoprenoids.tocopherol biosynthesis.hydroxyphenylpyruvate dioxygenase	at1g06570	HPD | PDS1 (PHYTOENE DESATURATION 1); 4-hydroxyphenylpyruvate dioxygenase	1,07
phenylpropanoids	at2g23910	cinnamoyl-CoA reductase-related	-1,36
N misc.alkaloid-like	at3g57030	strictosidine synthase family protein	-1,65
sulfurcontaining.glucosinolates.synthesis.aliphatic.branched-chain amino acid aminotransferase (BCAT/MAAT)	at3g49680	BCAT3 (BRANCHED-CHAIN AMINOTRANSFERASE 3); catalytic	1,45
flavonoids.chalcones	at5g05270	chalcone-flavanone isomerase family protein	-1,31
simple phenols	at5g05390	LAC12 (laccase 12)	-1,42
	at5g03260	LAC11 (laccase 11)	-1,35
	at2g38080	LAC4 | IRX12 (IRREGULAR XYLEM 12); laccase	-1,09
PEROXIDASES			
	at5g05340	peroxidase, putative	-1,3
	at1g71695	peroxidase 12 (PER12) (P12) (PRXR6)	-1,59
	at4g21960	PRXR1; electron carrier/ heme binding / peroxidase	-0,88
	at5g06730	peroxidase, putative	-1,4
MYB			
RNA.regulation of transcription.MYB domain transcription factor family	at1g34670	AtMYB93 (myb domain protein 93); DNA binding / transcription factor	-0,91
	at5g26660	ATMYB4, ATMYB86 (MYB DOMAIN PROTEIN 86); specific transcriptional repressor/ transcription repressor	-1,1

(continuação)

Categorie	ID *Arabidopsis*	Description	Fold Change
CELL WALL			
precursor synthesis.UGE	at1g12780	UGE1 (UDP-D-glucose/UDP-D-galactose 4-epimerase 1); UDP-glucose 4-epimerase/ protein dimerization	-1,08
cellulose synthesis	at5g03760	CSLA9, RAT4 | ATCSLA09; mannan synthase/ transferase, transferring glycosyl groups	1,33
cellulose synthesis.COBRA	at5g15630	COBL4, IRX6	-1,12
proteins.AGPs	at5g44130	FLA13 (FASCICLIN-LIKE ARABINOGALACTAN PROTEIN 13 PRECURSOR)	-2,07
degradation.cellulases and beta -1,4-glucanases	at2g32990	AtGH9B8 (Arabidopsis thaliana glycosyl hydrolase 9B8); catalytic/ hydrolase, hydrolyzing O-glycosyl compounds	-0,93
degradation.mannan-xylose-arabinose-fucose	at5g01930	(1-4)-beta-mannan endohydrolase, putative	-1,22
	at1g02640	BXL2 (BETA-XYLOSIDASE 2); hydrolase, hydrolyzing O-glycosyl compounds	-1,37
pectate lyases and polygalacturonases	at1g67750	pectate lyase family protein	-0,89
	at1g14420	AT59; lyase/ pectate lyase	-1,29
modification	at1g32170	XTH30 | XTR4 (XYLOGLUCAN ENDOTRANSGLYCOSYLASE 4); hydrolase, acting on glycosyl bonds / hydrolase, hydrolyzing O-glycosyl compounds / xyloglucan:xyloglucosyl transferase	-0,99
*pectin*esterases.PME*	at5g55590	QRT1 (QUARTET 1); pectinesterase	-1,3
PROTEIN DEGRADATION	at4g33490	aspartic-type endopeptidase	-1,5
Subtilases	at5g59810	SBT5.4; identical protein binding / serine-type endopeptidase	-0,91
cysteine protease	at4g35350	XCP1 (XYLEM CYSTEINE PEPTIDASE 1); cysteine-type endopeptidase/ cysteine-type peptidase	-1,2
	at5g04250	OTU-like cysteine protease family protein	-1,05
	at5g43060	cysteine proteinase, putative / thiol protease, putative	-0,8
serine protease	at5g47040	LON2 (LON PROTEASE 2); ATP binding / ATP-dependent peptidase/ nucleoside-triphosphatase/ nucleotide binding / serine-type endopeptidase/ serine-type peptidase	1,26
	at3g07990	SCPL27 (serine carboxypeptidase-like 27)	-1,44
AAA type	at5g40010	AATP1 (AAA-ATPase 1); ATP binding / ATPase/ nucleoside-triphosphatase/ nucleotide binding	-2,2
AAA type	at4g28000	ATP binding / ATPase/ nucleoside-triphosphatase/ nucleotide binding	-0,88

(continuação)

Categorie	ID *Arabidopsis*	Description	Fold Change
PROTEIN DEGRADATION			
ubiquitin.ubiquitin	at5g55160	SUMO2 (SMALL UBIQUITIN-LIKE MODIFIER 2); protein binding / protein tag	-0,8
ubiquitin.E2	at3g20060	UBC19 (ubiquitin-conjugating enzyme19); ubiquitin-protein ligase	1,57
	at1g14400	UBC1 (UBIQUITIN CARRIER PROTEIN 1); ubiquitin-protein ligase	0,8
ubiquitin.E3.RING	at1g18760	zinc finger (C3HC4-type RING finger) family protein	-1,33
	at1g68940	armadillo/beta-catenin repeat protein-related / U-box domain-containing protein	-0,86
	at4g35840	zinc finger (C3HC4-type RING finger) family protein	-0,78
	at2g17730	zinc finger (C3HC4-type RING finger) family protein	-0,85
	at1g47570	zinc finger (C3HC4-type RING finger) family protein	0,89
	at5g22000	RHF2A (RING-H2 GROUP F2A); protein binding / zinc ion binding	1,04
ubiquitin.E3.SCF.FBOX	at1g27340	F-box family protein	0,97
	at5g57360	LKP1, ADO1, FKL2 | ZTL (ZEITLUPE); protein binding / ubiquitin-protein ligase	1,06
	at1g67190	F-box family protein	0,95
ubiquitin.E3.BTB/POZ Cullin3.BTB/POZ	at3g48360	BT2 (BTB AND TAZ DOMAIN PROTEIN 2); protein binding / transcription factor/ transcription regulator	-1,56
ubiquitin protease	at5g57990	UBP23 (UBIQUITIN-SPECIFIC PROTEASE 23); ubiquitin thiolesterase/ ubiquitin-specific protease	1,19
PRS			
stress.biotic.PR-proteins	at1g50180	disease resistance protein (CC-NBS-LRR class), putative	-1,06
	at5g24090	acidic endochitinase (CHIB1)	-2,54
REDOX			
Thioredoxin	at4g03520	ATHM2; enzyme activator	-1,26
	at3g51030	ATTRX H1 | ATTRX1; oxidoreductase, acting on sulfur group of donors, disulfide as acceptor	-1,24
ascorbate and glutathione	at4g18260	cytochrome B561-related	-1,09
ascorbate and glutathione.ascorbate	at4g35970	APX5 (ASCORBATE PEROXIDASE 5); L-ascorbate peroxidase/ heme binding / peroxidase	-1,44

(continuação)

Categorie	ID *Arabidopsis*	Description	Fold Change
SIGNALLING			
sugar and nutrient physiology	at3g54040	photoassimilate-responsive protein-related	-1,66
receptor kinases.leucine rich repeat V	at4g03390	SRF3 (STRUBBELIG-RECEPTOR FAMILY 3); ATP binding / kinase/ protein serine/threonine kinase	-1
receptor kinases.leucine rich repeat XI	at5g56040	leucine-rich repeat protein kinase, putative	-1,02
receptor kinases.S-locus glycoprotein like	at4g21380	ARK3 (A. THALIANA RECEPTOR KINASE 3); kinase/ transmembrane receptor protein serine/threonine kinase	1,15
Calcium	at2g27030	CAM5 (CALMODULIN 5); calcium ion binding	-0,92
	at1g09210	calreticulin 2 (CRT2)	-1,05
	at4g20780	calcium-binding protein, putative	-1,86
G-proteins	at5g55190	RAN3 (RAN GTPASE 3); GTP binding / GTPase/ protein binding	-1,07
lipids	at1g60490	ATVPS34; 1-phosphatidylinositol-3-kinase/ binding / inositol or phosphatidylinositol kinase/ phosphotransferase, alcohol group as acceptor	0,92
light	at1g25540	PFT1 (PHYTOCHROME AND FLOWERING TIME 1); transcription coactivator	1,03
	at5g67385	protein binding / signal transducer	1,52
	at1g67310	calmodulin binding / transcription regulator	0,91
STRESS ABIOTIC	at4g11650	ATOSM34 (osmotin 34	-4,12
Heat	at5g42020	BIP, BIP2; ATP binding	-2,01
	at1g77020	DNAJ heat shock N-terminal domain-containing protein	-0,95
	at2g27140	heat shock family protein	-2,1
	at2g35795	DNAJ heat shock N-terminal domain-containing	-1,2
	at4g27670	HSP21 (HEAT SHOCK PROTEIN 21)	-1,69
	at3g62600	ATERDJ3B; heat shock protein binding / unfolded protein binding	-1
Cold	at3g17020	universal stress protein (USP) family protein	-1,21
drought/salt	at2g41430	LSR1, CID1 | ERD15 (EARLY RESPONSIVE TO DEHYDRATION 15); protein binding	-0,78
	at5g25610	RD22; nutrient reservoir	-1,24
	at3g05890	RCI2B (RARE-COLD-INDUCIBLE 2B)	-0,74
	at4g15910	ATDI21 (ARABIDOPSIS THALIANA DROUGHT-INDUCED 21)	-1,75

(conclusão)

Categorie	ID *Arabidopsis*	Description	Fold Change
STRESS ABIOTIC			
touch/wounding	at1g21610	wound-responsive family protein	1,06
Unspecified	at2g40330	Bet v I allergen family protein	-1,23
	at1g24020	MLP423 (MLP-LIKE PROTEIN 423)	-1,91
	at1g44760	universal stress protein (USP) family protein	1
	at1g20450	LTI29, LTI45, ERD10 (EARLY RESPONSIVE TO DEHYDRATION 10); actin binding	-1,27
	at3g05950	germin-like protein, putative	-1,15
WRKY			
RNA.regulation of transcription.WRKY domain transcription factor family	at3g04670	WRKY39; calmodulin binding	0,85
	at1g80840	WRKY40	-2,7
	at5g64810	WRKY51	-2,01
	at2g38470	WRKY33	-1,34

APÊNDICE E

Supplementary Table ST3. Primers used to validate the DEGs identified by RNAseq

Gene symbol	Sense (5´ to 3´)	Antisense (5´ to 3´)
OSM34	CTACGGACAGAACCCCAATACTCT	GAAGCTCAGCGGCACGTT
TIFY10B	GGAGGGAAAGGATTAACACCAA	GCCGAGCCACGACTTATTAT
PR4	GGCGACGTACAACATCTACAA	GCGGTCCAGTCATACTTCATC
Aco016970	ATGGTTCTCAAGGTCTCAATGG	CCTTCCAACGATGCGGATATAA
RTM2	TCTCCAAGTTCCCAGCAATTC	CGGCACTTCCATTCCTTTCT
BIP	GATGGGAAGGAGCCGAATAAG	GAGCAACATCCAGCAAGAGA
GSTF8	GATGTGCCTGTTTGAGAAGGA	ATCAGGAATACGCTTTGTACCC
SHY2	CCAGCATCTAAGGCACAAGTA	GCTAACTTTCACATACATCCCATTC
ERDJ3B	GACGAACATCCTGTGGAGATT	CCTTTCTTTGTGCTTTGGTAGAG
PDCB3	ACAACTACATCTACAGGGACTAATG	AGAGGGTGAGGGCTGAA
ARF4	GATGGGACGATAAGGTGGATTG	TTTGGAACTGGATGCTGAGAG
PRR5	GTACCACCCTCCAACGTAAAG	CAGAACATGAAGCACCAACATC
ACO4	CTTGGACTGGGAGAGCACCTT	TTCCTTCATCACGTTCCTGTAGTG
ELF7	ACTCTCCCATAACTCCAGAAGA	CTGAGTCTTGACCAACCATGAA
Actin	CGTTTGCGACAATGGAACTG	CGCTCTCGGTGCATCATCT
HSP70h (PMWaV-1)	GCAGGCGGTAGTAAACGAA	AAGTGCCTCCTCCGAAATC
HSP70h (PMWaV-2)	ACGGTACCAGCCGACTACA	CAGCGGTCGGTTCATTTAC